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(54) Title: METHOD FOR DOWN-REGULATING IL5 ACTIVITY
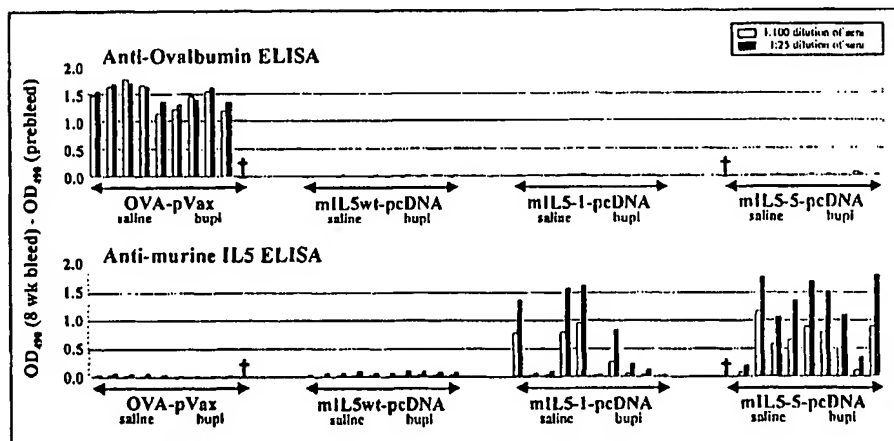
(57) Abstract

The present invention relates to improvements in therapy and prevention of conditions characterized by an elevated level of eosinophil leukocytes, i.e. conditions such as asthma and other chronic allergic diseases. A method is provided for down-regulating interleukin 5 (IL5) by enabling the production of antibodies against IL5 thereby reducing the level of activity of eosinophils. The invention also provides for methods of producing modified IL5 useful in this method as well as for the modified IL5 as such. Also encompassed by the present invention are nucleic acid fragments encoding modified IL5 as well as vectors incorporating these nucleic acid fragments and host cells and cell lines transformed therewith. The invention also provides for a method for the identification of IL5 analogues which are useful in the method of the invention as well as for compositions comprising modified IL5 or comprising nucleic acids encoding the IL5 analogues. The preferred embodiment of the present invention entails the use of variants of IL5, where foreign T helper epitopes are introduced so as to induce production of cross-reactive antibodies capable of binding to autologous IL5.

METHOD FOR DOWN-REGULATING IL5 ACTIVITY

FIELD OF THE INVENTION

5   The present invention relates to improvements in therapy and
prevention of conditions characterized by an elevated level of
eosinophil leukocytes, *i.e.* conditions such as asthma and
other chronic allergic diseases. More specifically, the pre-
sent invention provides a method for down-regulating inter-
10 leukin 5 (IL5) by enabling the production of antibodies
against IL5 thereby reducing the level of activity of eosino-
phils. The invention also provides for methods of producing
modified IL5 useful in this method as well as for the modified
IL5 as such. Also encompassed by the present invention are nu-
15 cleic acid fragments encoding modified IL5 as well as vectors
incorporating these nucleic acid fragments and host cells and
cell lines transformed therewith. The invention also provides
for a method for the identification of IL5 analogues which are
useful in the method of the invention as well as for composi-
20 tions comprising modified IL5 or comprising nucleic acids en-
coding the IL5 analogues.

BACKGROUND OF THE INVENTION
25
Asthma is a common disease of the airways, affecting about 10%
of the population. The present treatments is primarily based
on the administration of steroids and represents a market
value exceeding well over a billion dollars. For yet unknown
30 reasons the incidence and morbidity of asthmatics have in-
creased worldwide over the past two decades. Today, an im-
proved understanding of the immunological mechanisms involved
in asthmatic conditions combined with an explosive development
in biotechnology provides a new basis for the development of
35 alternative and perhaps better strategies for treatment.

A general feature in the pathogenesis of asthma and other
chronic allergic diseases has proven to be elevated numbers of

eosinophils, especially in the bronchial mucosa of the lungs.
Upon activation eosinophils secrete a number of mediators that
are actively involved in the inflammatory airway response. In
the activation of eosinophils, interleukin 5 (IL5) plays an
5 important role.

IL5 is a cytokine found in many mammalian species and among
others both the human and the murine gene for IL5 have been
cloned (Tanabe et al., 1987, Campbell et al., 1988). The human
10 gene consists of four exons with three introns positioned at
chromosome 5 and codes for a 134 amino acid residue precursor,
including a 19 amino acid N-terminal leader sequence which has
the amino acid sequence set forth in SEQ ID NO: 62.
Posttranslational cleavage generates the mature 115 amino acid
15 residue protein (SEQ ID NO: 1). The murine IL5 (mIL5) gene
similarly codes for a 133 amino acid residue pre-cursor with a
20 amino acid leader sequence which has the amino acid se-
quence set forth in SEQ ID NO: 64. The processed mature mIL5
is thus 113 amino acid residues long (SEQ ID NO: 12), missing
20 two N-terminal amino acid residues by alignment with human
IL5. The amino acid sequences of hIL5 and mIL5 are 70% iden-
tical compared to 77% at nucleotide level of the coding re-
gions (Azuma et al., 1986). Higher similarity was reported
within human primates; 99% identity is reported for the coding
25 regions of the human and the Rhesus monkey nucleotide se-
quences (Villinger et al., 1995).

The human amino acid sequence has two potential N-glycosyla-
tion sites and the murine three. Human IL5 has been shown to
30 be both N-glycosylated as well as O-glycosylated at Thr 3.
Studies of hIL5 has demonstrated that the glycosylation is not
necessary for the biological activity even though the stabil-
ity seems to be affected by de-glycosylation (Tominaga et al.,
1990; Kodama et al., 1993).

35

3

Structure of IL5

The active IL5 is a homo-dimer and the 3-dimensional structure
of recombinant hIL5 has been determined by X-ray crystallogra-
5 phy (Milburn et al., 1993). The 2 monomers are organised in an
antiparallel manner and covalently bound by two interchain di-
sulfide bridges (44-87' and 87-44'), thus engaging all 4 cys-
teines of the 2 monomers.

10 The secondary structure of the monomers consists of 4 α-
helices (A-D) intermitted by 3 linking regions (loops)
including two short stretches of β-sheets. This 4α helix bundle
is known as the "common cytokine fold", which has also been
reported for IL-2, IL-4, GM-CSF, and M-CSF. But all these are
15 monomers and the homodimer-structure in which the D-helix
completes the 4α helix motif of the opposite monomer is unique
to IL5.

The native monomers alone has been shown to be biologically
20 inactive (for reviews see Callard & Gearing, 1994; Takutsu et
al., 1997). It is nevertheless possible to produce a modified
recombinant biologically active monomer by inserting 8 addi-
tional amino acid residues in loop 3, connecting the helices C
and D. This enables helix D to complete the 4 helix structure
25 within one polypeptide chain and thus enable the monomer to
interact with its receptor (Dickason & Huston, 1996; Dickason
et al., 1996).

The IL5 receptor is primarily present on eosinophils and it is
30 composed of an α-chain and a β-chain. The α-chain of the
receptor is specific for IL5 and the β-chain, which assure
high-affinity binding and signal transduction, is shared with
the hetero-dimer receptors for IL-3 and GM-CSF. The sharing of
a receptor component could be the reason for the cross-
35 competition seen between IL5, IL-3 and GM-CSF (for review, see
Lopez et al., 1992). However, it was recently demonstrated
that the regulation of the IL5R is distinct from the
regulation of the IL-3R and the GM-CSFR, further indicating a

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highly specialised role of IL5 in the regulation of the
eosinophilic response (Wang et al., 1998).

5 The C-terminal part of IL5 seems to be important in both bin-
ding to the IL5R and for the biological activity, since remo-
val of more than two C-terminal amino acid residues results in
a decline in both the binding affinity to the IL5 R and in the
biological activity in an IL5 bioassay (Proudfoot et al.,
1996). Other residues have also been found to be important for
10 binding to the receptor, such as Glu12, which is involved in
binding to the β-chain, while the Arg90 and Glu109 residues are
involved in the binding to the α-chain of the receptor. In
general, binding to the IL5R seems to occur in regions
overlapping helices A and D, where helix D is primarily
15 responsible for the binding to the specific IL5R α-chain
(Graber et al., 1995; Takastsu et al., 1997).

IL5's homology to other proteins

20 The two 4-helix domain motifs seen in the homodimer has stri-
kingly similar secondary and tertiary structure as compared to
the cytokine fold found in GM-CSF and M-CSF, IL-2, IL-4 and
human and porcine growth hormone (Milburn et al., 1993). How-
ever, even though striking similarities are also observed in
25 the intron/exon organisation and position of cysteines (Tanabe
et al., 1987; Cambell et al., 1988) suggesting a phylogenetic
relationship with IL-2, IL-4 and GM-CSF, no significant homo-
logy with any of these or other cytokines is observed from the
amino acid sequence.
30
Biological activity of IL5

IL5 is mainly secreted by fully differentiated Th2 cells, mast
cells and eosinophils (Cousins et al., 1994; Takutsu et al.,
35 1997). It has been shown to act on eosinophils, basophiles,
cytotoxic T lymphocytes and on murine B cells (Callard & Gear-
ing, 1994; Takutsu et al., 1997). The effects of IL5 on human
B cells are still a matter of controversy. Augmentation of im-

munoglobulin synthesis under certain circumstances and binding
to a variety of human B cell lines have been demonstrated.
Even though mRNA for the hIL5R has been found in human B-
cells, the actual presence of the receptor on these cells has
5 still to be verified (Baumann & Paul, 1997; Huston et al.,
1996).

The actions of IL5 on eosinophils include chemotaxis, enhanced
adhesion to endothelial cells, activation and terminal diffe-
10 rentiation of the cells. Furthermore it has been demonstrated
that IL5 prevents mature eosinophils from apoptosis (Yamaguchi
et al., 1991). These findings have contributed to the present
concept of IL5 as being the most important cytokine for eosi-
nophil differentiation (Corrigan & Kay, 1996; Karlen et al.,
15 1998).

Physiologically, IL5 and its associated eosinophil activation
is considered to serve a protective role against helminthic
infections and possibly against certain tumours, since these
20 diseases are typically accompanied by peripheral blood
eosinophilia (Takutsu et al., 1997; Sanderson et al., 1992).
It is, however, somewhat speculative as in two studies the
authors failed to show any effect beside eosinophil down-
regulation following administration of antibodies against IL5
25 on the immunity (e.g. IgE levels) against Nippostrongylus
braziliensis or Schistosoma mansoni in mice infected with
these parasites (Sher et al., 1990; Coffman et al., 1989).

IL5 transgenic and "knock-out" animals

30

Studies of transgenic mice expressing IL5 or knock-out mice
deficient for IL5 have given further knowledge of the physio-
logical role of IL5.

35 Several IL5 transgenic mice have been reported:

A transgenic mouse expressing the IL5 gene in T cells was re-
ported to have an increased white blood cell level characte-

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rised by expansion of B220+ B lymphocytes and profound eosino-
philia. This was accompanied by a massive peritoneal cavity
cell exudate dominated by eosinophils and infiltration of
eosinophils in nearly all organ systems (Lee et al., 1997a).

5

Another transgenic mouse, expressing the IL5 gene under con-
trol of a metallothionin promoter was characterised by an in-
crease in the serum levels of IgM and IgA, a massive eosino-
philia in peripheral blood and many other organs accompanied
10 by the expansion of a distinctive CD5+ B cell population,
which produce auto-antibodies (Tominaga et al., 1991).

A third study involved a transgenic mouse constitutively ex-
pressing IL5 in the lungs. These animals developed pathophysi-
15 ological changes resembling those of human asthma, including
eosinophil invasion of peribronchial spaces, epithelial hyper-
trophy and increased mucus production. Furthermore, develop-
ment of airway hyper responsiveness was seen in the absence of
antigens (Lee et al., 1997b).

20

IL5-deficient mice ('knock-out' mice) have also been studied.
These mice (C57BL/6) have no obvious signs of disease and are
fertile. The immunoglobulin levels and the specific antibody
responses to DNP-OVA were normal. Basal levels of eosinophils
25 are produced, but are 2-3 times lower than in control animals,
indicating that eosinophils can be produced in the complete
absence of IL5. When these mice were infected with Mesoces-
toides corti the eosinophilia normally seen was abolished and
this absence of eosinophilia did not affect the worm burden
30 produced by this parasite (Kopf et al., 1996).

In a study by Foster et al. (1996), the effect of IL5 knock-
out on a common model of atopic airway inflammation was inves-
tigated. Sensitisation and aerosol challenge of mice with
35 ovalbumin normally result in airway eosinophilia, airway hy-
perreactivity to β-methacholin and extensive lung damage analo-
gous to that seen in asthma. In the IL5 deficient mice the
eosinophilia, airway hyperreactivity and lung damage were

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abolished. When IL5 expression in these mice was reconsti-
tuted, the aero-allergen induced eosinophilia and airway dys-
function were restored.

5 **Pathophysiologic role of IL5**

Asthma affect about 10% of the population worldwide and for
yet unknown reasons the incidence and morbidity have increased
over the past two decades (Ortega & Busse, 1997). It is a
10 chronic airway disease characterised by recurrent and usually
reversible air flow obstruction, inflammation and hyper re-
sponsiveness (Moxam and Costello, 1990). This produces symp-
toms of wheezing and breathlessness, which in severe cases can
be fatal.
15
The animal experiments referred to above using transgenic mice
constitutively expressing IL5 in the lungs (Lee et al.,.
1997a) and the IL5 deficient "knock-out" mice (Foster et al.,
1996) strongly implicate a crucial role of IL5 in the patho-
20 genesis of asthma. Further evidence supporting this can be de-
duced from several studies including asthmatic individuals.

Eosinophilia has been identified in bronchoalveolar lavage
(BAL) fluid and in bronchial mucosal biopsies of subjects with
25 asthma and correlates with disease severity. Several eosino-
phil products have been identified in the BAL fluid of pa-
tients with asthma and numbers of peripheral blood eosinophils
correlate with asthma severity (Ortega & Busse 1997).

30 IL5 serum concentration was found to be elevated (median con-
centration 150 pg/ml) in 15 out of 29 patients with chronic
severe asthma as compared to control subjects (Alexander et
al., 1994).

35 In another study involving both non-atopic and atopic asthma-
tics, it was found that an enhanced IL5 production by helper T
cells seems to cause the eosinophilic inflammation of both
atopic and non-atopic asthma (Mori et al., 1997).

8

Other results also indicate that IL5 has a distinct role in
other atopic diseases. Allergen induced systemic episodes in
individuals with allergic rhinitis has recently been shown to
5 correlate to allergen induced IL5 synthesis rather than IgE
(Ohashi et al., 1998). The correlation of atopic reactions is
also demonstrated in a study by Barata et al. (1998) in which
a significant expression of IL5 by T-cells in a cutaneous late
phase reaction is demonstrated.
10   ,

These and other results have led several authors as Corrigan &
Kay (1996), Danzig & Cuss (1997) to identify and recommend IL5
as a primary target in the development of a better treatment
for asthma and atopic diseases involving eosinophilic inflam-
15 mation. Chronic tissue damaging hypereosinophilia induced by
parasitic infection, topical pulmonary eosinophilia and hy-
pereosinophilic syndrome are examples of other pathogenic con-
ditions that could be addressed by IL5 down regulation.

20 *In vivo* **demonstration of the role of IL5**

In several studies with rodent models of asthma it has been
shown that treatment with monoclonal antibodies against IL5
(anti-IL5 mAb) results in dose-related inhibition of eosino-
25 philia, as compared to non-treated controls (Nagai et al.,
1993a & b; Chand et al., 1992; Coeffier et al., 1994; Kung et
al., 1995; Underwood et al., 1996). In the study by Nagai et
al. (1993a) the effect was also observed by treating the sen-
sitised Balb/c mice with soluble IL5 receptor α.
30

In one study with Balb/c mice (*Hamelmann et al.*, 1997) and
four studies with guinea pigs it was additionally shown that
anti-IL5 mAb could inhibit airway hyperreactivity elicited
with various substances in antigen sensitised animals (Mauser
35 et al., 1993; Akutsu et al., 1995; van Oosterhout et al., 1995
& 1993). In some of the studies beneficial effects (cf. table
1) of the anti-IL5 mAb treatment were also observed micro-
scopically (Mauser et al., 1993; Akutsu et al., 1995; Kung et

9

al., 1995). Importantly, in the study by Kung *et al.* (1995) a reduction of pulmonary inflammation in B6D2F1 mice was seen both when anti-IL5 mAb was administered hours before antigen challenge and also when administered up to five days after an-

5 tigen challenge, indicating that the effect of anti-IL5 mAb may be both prophylactic and therapeutic for airway inflammation. This effect, however, was not observed by Underwood et *al.* when guinea pigs were given anti-IL5 mAb two hours after antigen challenge (Underwood et *al.*, 1996).

10

In a study using a monkey model of asthma, Mauser et al. (1995) reported an inhibition of airway hyper reactivity after antigen challenge, when rat anti mouse-IL5 mAb was given 1 hour before antigen challenge. In addition, there was 75% re-

15 duction in the number of eosinophils in bronchoalveolar lavage (BAL) of antibody treated animals, as compared to non-treated controls. The effects on eosinophilia and hyperresponsiveness of anti-IL5 mAb was seen for up to three months after treatment (Mauser et *al.*, 1995). Regarding allergic hyperrespon-

20 siveness, the results from studies by Nagai *et al.* (1993a and 1993b) document no reduction in hyperresponsiveness in conjunction to a reduction of eosinophil numbers in BAL.

All anti-IL5 mAb *in vivo* experiments mentioned so far have

25 been done with rat-anti-mouse monoclonal antibodies. Egan et *al.* (1995) have reported experiments using humanised rat-anti-human IL5 monoclonal antibodies, called Sch 55700. These mAbs, inhibited lung lavage eosinophilia by 75% at a dose of 0,3 mg/kg when administered to sensitised monkeys. When Sch 55700

30 was given at 1 mg/kg in allergic mice, inhibition of airway eosinophilia was also observed.

Treatment of asthma at present and in the future

35 The current treatment of asthma is, as mentioned, corticosteroids which, by their anti-inflammatory action, are the most powerful drugs. Besides this, β_2 agonists and methyl xanthine derivatives which all cause bronchodilation, and disodium

chromoglycate which 'stabilises' mast cells, thereby prevent-
ing mediator release, all have proven beneficial in asthma pa-
tients (Ortega & Busse 1997).

5 Future treatment of asthma may as discussed above include
anti-IL5 mAbs. Celltech in corporation with Schering Plough
have anti-IL5 mAb in phase I clinical trial for treatment of
asthma. However, treatment with monoclonal antibodies entails
a number of drawbacks. First of all, the development and pro-
10 duction costs for a safe mAB (e.g. a humanised mAB) are very
high, resulting in an expensive therapeutic product for the
end user. Second, mABs have the disadvantageous characteristic
seen from a patient point of view that they have to be admi-
nistered with relatively short intervals. Third, by nature
15 mABs exhibit a narrow specificity against one single epitope
of the antigen. And, finally, mABs (even humanised) are immu-
nogenic, leading to an increasingly fast inactivation of ad-
ministered antibodies as treatment progresses over time.

20 Also use of antisense IL5 oligonucleotides for antisense the-
rapy has been suggested by the company Hybridon for the treat-
ment of asthma, allergies and inflammation. However, the an-
tisense technology has proven to be technically difficult and,
in fact, conclusive evidence of the feasibility of antisense
25 therapy in humans has not yet been established.

Finally, WO 97/45448 (Bresagen Limited / Medvet Science) pro-
poses the use of "modified and variant forms of IL5 molecules
capable of antagonising the activity of IL5" in ameliorating,
30 abating or otherwise reducing the aberrant effects caused by
native or mutant forms of IL5. The antagonizing effect is re-
ported to be the result of the variant forms of IL5 binding to
the low affinity α chain of IL5R but not to the high affinity
receptors; in this way the variants compete with IL5 for bin-
35 ding to its receptors without exerting the physiological ef-
fects of IL5.

Other atopic diseases involving eosinophilic inflammation are treated with either the symptomatica mentioned for asthma or immune therapy (IT) using hyposensitization with allergen extracts. The latter type of treatment is known to be effective
5 against allergies against one or a few antigens, whereas IT is not feasible in the treatment of multiple allergies. Furthermore, the time scale for obtaining clinical improvement in patients susceptible to treatment is very long for conventional IT.
10

Thus, in spite of existing and possible future therapies for chronic allergic diseases such as asthma, there is a definite need for alternative ways of treating and ameliorating this and other chronic allergic diseases.
15

OBJECT OF THE INVENTION

The object of the present invention is to provide novel thera-
20 pies against chronic allergic conditions (such as asthma) characterized by eosinophilia. A further object is to develop an autovaccine against IL5, in order to obtain a novel treatment for asthma and for other pathological disorders involving chronic airway inflammation.
25

SUMMARY OF THE INVENTION

The T-cell derived cytokine IL5 has, as mentioned above, a
30 crucial role in orchestrating the eosinophilic response, affecting both the production, the localisation and the activation of eosinophils. As IL5 has not otherwise been reported to have a central role in the development of a protective immune response, this particular cytokine is in the opinion of the
35 inventors an attractive therapeutic target for the treatment of asthma.

The general aim according to the present invention is to de-
crease the pathogenic levels of eosinophils in the airways of
the asthma patient by down-regulating of the IL5 levels, since
eosinophils depend on IL5 for attraction and activation. The
5 result of a decreased eosinophil number in the airway mucosa
would be a concomitant decrease in the airway inflammation,
corresponding to a clinical improvement in the asthmatic pa-
tient.

10 The potential effect of such an approach has already been de-
monstrated in studies using anti IL5 monoclonal antibodies in
animal models of airway inflammation, cf. the "PREAMBLE TO EX-
AMPLES".

15 This current invention, however, takes the results obtained
through passive immunisation one step further by using the ap-
proach of generating an active immune response through the
concept of autovaccination. To the best of the inventor's
knowledge, such an approach has never been suggested before.
20
The advantage of treating asthmatics with an IL5 autovaccine,
as compared to current treatment with corticosteroids etc., is
a reduction and/or elimination of side effects and most likely
a better effect in terms of duration. When compared to
25 anti-IL5 mAbs, the effect of an induced polyclonal Ab response
is expected to be superior to passively injected monoclonal
immunoglobulins since the polyclonal response has a broader
specificity. Improvements with respect to administration regi-
men are also expected (since effective autovaccines described
30 herein typically would require a maximum of 2-6 administra-
tions per year).

When compared to hyposensitization, the present invention of-
fers the attractive aspect of being non-specific; this is es-
35 pecially relevant when dealing with multi-allergic patients.

Thus, in its broadest and most general scope, the present in-
vention relates to a method for in vivo down-regulation of in-

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terleukin 5 (IL5) activity in an animal, including a human be-
ing, the method comprising effecting presentation to the ani-
mal's immune system of an immunologically effective amount of

-       at least one IL5 polypeptide or subsequence thereof which
5   has been formulated so that immunization of the animal
         with the IL5 polypeptide or subsequence thereof induces
         production of antibodies against the IL5 polypeptide,
         and/or
-       at least one IL5 analogue wherein is introduced at least
10   one modification in the IL5 amino acid sequence which has
         as a result that immunization of the animal with the ana-
         logue induces production of antibodies against the IL5
         polypeptide.

15 The most attractive aspect of this approach is that e.g.
    asthma can be controlled by periodic but not very frequent im-
    munizations, in contrast to a therapeutic approach which in-
    volves administration of anti-IL5 or molecules having a bind-
    ing affinity to IL5 analogous therewith. It is expected that
20 1-4 annual injections with an immunogenic composition accord-
    ing to the invention will be sufficient to obtain the desired
    effect, whereas administration of other inhibitors of IL5 ac-
    tivity does or will require daily, or at least weekly, admini-
    strations.
25
    The invention also relates to IL5 analogues as well as to nu-
    cleic acid fragments encoding a subset of these. Also immuno-
    genic compositions comprising the analogues or the nucleic
    acid fragments are part of the invention.
30
    The invention also relates to a method of identifying ana-
    logues of IL5 as well as a method for preparing a composition
    comprising the IL5 analogues.

35

LEGENDS TO THE FIGURES

Fig. 1:   The amino acid sequence of the mature human IL5
           (SEQ ID NO: 1). The aligned murine sequence is in-
5   cluded (SEQ ID NO: 12), but only positions that dif-
           fer from the human sequence are displayed. The two
           "*"s indicate the missing N-terminal residues of the
           murine IL5. The N-glycosylation positions are marked
           with double underlining, the O-glycosylated threo-
10   nines of human IL5 are given in italics, and the
           cysteines in bold.

Fig. 2:   The dimer and monomer structures of human IL5.
           A: Dimer structure of hIL5. The structure has only
15   been obtained for residues 5-112, which means that
           the O-glycosylation site at Thr3 is not included.
           B: The same structure as in A, with the assignment
           of the helices (A-D and A'-D').
           C: The monomer hIL5 with the amino acid residues
20   differing from the mIL5 shown in light grey.

Fig. 3:   The aligned mature human IL5 (hIL5) and murine IL5
           (mIL5) amino acid sequences (SEQ ID NOs: 1 and 12)
           with indications of suitable substitution regions.
25   The 4 α-helices A-D are surrounded by solid-line
           boxes, the β-sheets are double underlined and the po-
           sitions of the two cysteines are marked with "▼".
           Identical residues in the two sequences are marked
           with "-" and non-identical residues with "*". Loop 1
30   spans between helices A and B, Loop 2 spans between
           helices B and C, and loop 3 spans between loops C
           and D. Amino acid sequences to be substituted with
           foreign T_H epitope containing peptides are marked in
           bold; one such sequence is surrounded by a dot-lined
35   box because of residues overlapping with those sub-
           stituted in a different construct. The amino acid
           sequences of 10 constructs (5 derived from human and

15

5 derived from murine IL5) are set forth in SEQ ID
NOs: 2-11 and 13-22.

Fig. 4:   ELISA results of DNA immunization testing two mIL5
            autovaccine DNA vaccines.
            Mice were DNA vaccinated with naked plasmid DNA en-
            coding either ovalbumin, mIL5wt, mIL5.1 or mIL5.5.
            Sera obtained at day 77 were tested for reactivity
            against ovalbumin and murine IL5. Polystyrene micro-
            titer plates (Maxisorp, Nunc) were coated with oval-
            bumin (1 µg/well, Sigma) or purified recombinant mur-
            ine IL5 (0.1 µg/well, E1320). The reactivities of di-
            luted sera added to the wells were visualised using a
            goat anti-mouse secondary antibody. OD490 readings of
            the pre-bleeds were subtracted from the OD490 read-
            ings of the test samples, and the resulting values
            were presented for each individual mouse as bars. The
            OD490 readings of the pre-bleeds (in 1:25 dilution)
            were ranging from 0.025-0.034. Crucifixes indicate
            dead animals.

Fig. 5:   Schematic representation of murine IL5 based autovac-
            cine constructs.
            The top figure represents murine wild-type IL5 mono-
            mer with helices A-C, loops 1-3 and the flexible C-
            terminal region. Remaining figures represent diffe-
            rent autovaccine constructs having in-substitutions
            of the tetanus toxoid epitopes P2 and P30 in various
            positions. Specific constructs are detailed in the
            Examples.

DETAILED DISCLOSURE OF THE INVENTION

Definitions

5 In the following, a number of terms used in the present speci-
   fication and claims will be defined and explained in detail in
   order to clarify the metes and bounds of the invention.

   The terms "T-lymphocyte" and "T-cell" will be used inter-
10 changeably for lymphocytes of thymic origin which are respon-
   sible for various cell mediated immune responses as well as
   for helper activity in the humeral immune response. Likewise,
   the terms "B-lymphocyte" and "B-cell" will be used inter-
   changeably for antibody-producing lymphocytes.

15
   An "IL5 polypeptide" is herein intended to denote polypeptides
   having the amino acid sequence of the above-discussed IL5 pro-
   teins derived from humans and mice (or truncates thereof sha-
   ring a substantial amount of B-cell epitopes with intact IL5),
20 but also polypeptides having the amino acid sequence identical
   to xeno-analogues of these two proteins isolated from other
   species are embraced by the term. Also unglycosylated forms of
   IL5 which are prepared in prokaryotic system are included
   within the boundaries of the term as are forms having varying
25 glycosylation patterns due to the use of e.g. yeasts or other
   non-mammalian eukaryotic expression systems. It should, how-
   ever, be noted that when using the term "an IL5 polypeptide"
   it is intended that the polypeptide in question is normally
   non-immunogenic when presented to the animal to be treated. In
30 other words, the IL5 polypeptide is a self-protein or is a
   xeno-analogue of such a self-protein which will not normally
   give rise to an immune response against IL5 of the animal in
   question.

35 An "IL5 analogue" is an IL5 polypeptide which has been sub-
   jected to changes in its primary structure. Such a change can
   e.g. be in the form of fusion of an IL5 polypeptide to a suit-
   able fusion partner (i.e. a change in primary structure exclu-

sively involving C- and/or N-terminal additions of amino acid residues) and/or it can be in the form of insertions and/or deletions and/or substitutions in the IL5 polypeptide's amino acid sequence. Also encompassed by the term are derivatized
5 IL5 molecules, cf. the discussion below of modifications of IL5.

It should be noted that the use as a vaccine in a human of e.g. a canine analogue of human IL5 can be imagined to produce
10 the desired immunity against IL5. Such use of an xeno-analogue for immunization is also considered to be an "IL5 analogue" as defined above.

When using the abbreviation "IL5" herein, this is intended as
15 a reference to the amino acid sequence of mature, wildtype IL5 (also denoted "IL5m" and "IL5wt" herein). Mature human IL5 is denoted hIL5, hIL5m or hIL5wt, and murine mature IL5 is denoted mIL5, mIL5m, or mIL5wt. In cases where a DNA construct includes information encoding a leader sequence or other mate-
20 rial, this will normally be clear from the context.

The term "polypeptide" is in the present context intended to mean both short peptides of from 2 to 10 amino acid residues, oligopeptides of from 11 to 100 amino acid residues, and poly-
25 peptides of more than 100 amino acid residues. Furthermore, the term is also intended to include proteins, i.e. functional biomolecules comprising at least one polypeptide; when comprising at least two polypeptides, these may form complexes, be covalently linked, or may be non-covalently linked. The
30 polypeptide(s) in a protein can be glycosylated and/or lipidated and/or comprise prosthetic groups.

The term "subsequence" means any consecutive stretch of at least 3 amino acids or, when relevant, of at least 3 nucleo-
35 tides, derived directly from a naturally occurring IL5 amino acid sequence or nucleic acid sequence, respectively.

The term "animal" is in the present context in general in-
tended to denote an animal species (preferably mammalian),
such as *Homo sapiens*, *Canis domesticus*, etc. and not just one
single animal. However, the term also denotes a population of
5   such an animal species, since it is important that the indi-
viduals immunized according to the method of the invention all
harbour substantially the same IL5 allowing for immunization
of the animals with the same immunogen(s). If, for instance,
genetic variants of IL5 exists in different human population
10 it may be necessary to use different immunogens in these dif-
ferent populations in order to be able to break the autotole-
rance towards IL5 in each population. It will be clear to the
skilled person that an animal in the present context is a liv-
ing being which has an immune system. It is preferred that the
15 animal is a vertebrate, such as a mammal.

By the term "*in vivo* down-regulation of IL5 activity" is
herein meant reduction in the living organism of the number of
interactions between IL5 and its receptors (or between IL5 and
20 other possible biologically important binding partners for
this molecule). The down-regulation can be obtained by means
of several mechanisms: Of these, simple interference with the
active site in IL5 by antibody binding is the most simple.
However, it is also within the scope of the present invention
25 that the antibody binding results in removal of IL5 by scaven-
ger cells (such as macrophages and other phagocytic cells).

The expression "effecting presentation ... to the immune sys-
tem" is intended to denote that the animal's immune system is
30 subjected to an immunogenic challenge in a controlled manner.
As will appear from the disclosure below, such challenge of
the immune system can be effected in a number of ways of which
the most important are vaccination with polypeptide containing
"pharmaccines" (*i.e.* a vaccine which is administered to treat
35 or ameliorate ongoing disease) or nucleic acid "pharmaccine"
vaccination. The important result to achieve is that immune
competent cells in the animal are confronted with the antigen
in an immunologically effective manner, whereas the precise

19

mode of achieving this result is of less importance to the in-
ventive idea underlying the present invention.

The term "immunogenically effective amount" has its usual
5 meaning in the art, *i.e.* an amount of an immunogen which is
capable of inducing an immune response which significantly en-
gages pathogenic agents which share immunological features
with the immunogen.

10 When using the expression that the IL5 has been "modified" is
herein meant a chemical modification of the polypeptide which
constitutes the backbone of IL5. Such a modification can e.g.
be derivatization (e.g. alkylation, acylation, esterification
etc.) of certain amino acid residues in the IL5 sequence, but
15 as will be appreciated from the disclosure below, the pre-
ferred modifications comprise changes of (or additions to) the
primary structure of the IL5 amino acid sequence.

When discussing "autotolerance towards IL5" it is understood
20 that since IL5 is a self-protein in the population to be vac-
cinated, normal individuals in the population do not mount an
immune response against IL5; it cannot be excluded, though,
that occasional individuals in an animal population might be
able to produce antibodies against native IL5, e.g. as part of
25 an autoimmune disorder. At any rate, an animal will normally
only be autotolerant towards its own IL5, but it cannot be ex-
cluded that IL5 analogues derived from other animal species or
from a population having a different IL5 phenotype would also
be tolerated by said animal.
30
A "foreign T-cell epitope" (or: "foreign T-lymphocyte epi-
tope") is a peptide which is able to bind to an MHC molecule
and which stimulates T-cells in an animal species. Preferred
foreign T-cell epitopes in the invention are "promiscuous"
35 epitopes, *i.e.* epitopes which bind to a substantial fraction
of a particular class of MHC molecules in an animal species or
population. Only a very limited number of such promiscuous T-
cell epitopes are known, and they will be discussed in detail

below. It should be noted that in order for the immunogens
which are used according to the present invention to be effec-
tive in as large a fraction of an animal population as possi-
ble, it may be necessary to 1) insert several foreign T-cell

5   epitopes in the same IL5 analogue or 2) prepare several IL5
analogues wherein each analogue has a different promiscuous
epitope inserted. It should be noted also that the concept of
foreign T-cell epitopes also encompasses use of cryptic T-cell
epitopes, *i.e.* epitopes which are derived from a self-protein

10 and which only exerts immunogenic behaviour when existing in
isolated form without being part of the self-protein in ques-
tion.

A "foreign T helper lymphocyte epitope" (a foreign T_H epitope)

15 is a foreign T cell epitope which binds an MHC Class II
molecule and can be presented on the surface of an antigen
presenting cell (APC) bound to the MHC Class II molecule.

A "functional part" of a (bio)molecule is in the present con-

20 text intended to mean the part of the molecule which is re-
sponsible for at least one of the biochemical or physiological
effects exerted by the molecule. It is well-known in the art
that many enzymes and other effector molecules have an active
site which is responsible for the effects exerted by the mole-

25 cule in question. Other parts of the molecule may serve a sta-
bilizing or solubility enhancing purpose and can therefore be
left out if these purposes are not of relevance in the context
of a certain embodiment of the present invention. For instance
it is possible to use certain other cytokines as a modifying

30 moiety in IL5 (cf. the detailed discussion below), and in such
a case, the issue of stability may be irrelevant since the
coupling to IL5 provides the stability necessary.

The term "adjuvant" has its usual meaning in the art of vac-

35 cine technology, *i.e.* a substance or a composition of matter
which is 1) not in itself capable of mounting a specific im-
mune response against the immunogen of the vaccine, but which
is 2) nevertheless capable of enhancing the immune response

against the immunogen. Or, in other words, vaccination with
the adjuvant alone does not provide an immune response against
the immunogen, vaccination with the immunogen may or may not
give rise to an immune response against the immunogen, but the
5 combination of vaccination with immunogen and adjuvant induces
an immune response against the immunogen which is stronger
than that induced by the immunogen alone.

"Targeting" of a molecule is in the present context intended
10 to denote the situation where a molecule upon introduction in
the animal will appear preferentially in certain tissue(s) or
will be preferentially associated with certain cells or cell
types. The effect can be accomplished in a number of ways in-
cluding formulation of the molecule in composition facilita-
15 ting targeting or by introduction in the molecule of groups
which facilitates targeting. These issues will be discussed in
detail below.

"Stimulation of the immune system" means that a substance or
20 composition of matter exhibits a general, non-specific immu-
nostimulatory effect. A number of adjuvants and putative adju-
vants (such as certain cytokines) share the ability to stimu-
late the immune system. The result of using an immunostimula-
ting agent is an increased "alertness" of the immune system
25 meaning that simultaneous or subsequent immunization with an
immunogen induces a significantly more effective immune re-
sponse compared to isolated use of the immunogen

Preferred embodiments of IL5 activity down-regulation

30
It is preferred that the IL5 polypeptide used as an immunogen
in the method of the invention is a modified molecule wherein
at least one change is present in the IL5 amino acid sequence,
since the chances of obtaining the all-important breaking of
35 autotolerance towards IL5 is greatly facilitated that way. It
should be noted that this does not exclude the possibility of
using such a modified IL5 in formulations which further fa-
cilitate the breaking of autotolerance against IL5, e.g. for-

mulations containing certain adjuvants discussed in detail be-
low.

It has been shown (in Dalum I *et al.*, 1996, J. Immunol. 157:
5 4796-4804) that potentially self-reactive B-lymphocytes recog-
nizing self-proteins are physiologically present in normal in-
dividuals. However, in order for these B-lymphocytes to be in-
duced to actually produce antibodies reactive with the rele-
vant self-proteins, assistance is needed from cytokine produ-
10 cing T-helper lymphocytes (T_H-cells or T_H-lymphocytes). Nor-
mally this help is not provided because T-lymphocytes in gen-
eral do not recognize T-cell epitopes derived from self-pro-
teins when presented by antigen presenting cells (APCs). How-
ever, by providing an element of "foreignness" in a self-pro-
15 tein (*i.e.* by introducing an immunologically significant modi-
fication), T-cells recognizing the foreign element are acti-
vated upon recognizing the foreign epitope on an APC (such as,
initially, a mononuclear cell). Polyclonal B-lymphocytes
(which are also specialised APCs) capable of recognising
20 self-epitopes on the modified self-protein also internalise
the antigen and subsequently presents the foreign T-cell epi-
tope(s) thereof, and the activated T-lymphocytes subsequently
provide cytokine help to these self-reactive polyclonal B-lym-
phocytes. Since the antibodies produced by these polyclonal B-
25 lymphocytes are reactive with different epitopes on the modi-
fied polypeptide, including those which are also present in
the native polypeptide, an antibody cross-reactive with the
non-modified self-protein is induced. In conclusion, the
T-lymphocytes can be led to act as if the population of poly-
30 clonal B-lymphocytes have recognised an entirely foreign anti-
gen, whereas in fact only the inserted epitope(s) is/are for-
eign to the host. In this way, antibodies capable of
cross-reacting with non-modified self-antigens are induced.

35 Several ways of modifying a peptide self-antigen in order to
obtain breaking of autotolerance are known in the art. Hence,
according to the invention, the modification can include that

- at least one foreign T-cell epitope is introduced, and/or
- at least one first moiety is introduced which effects targeting of the modified molecule to an antigen presenting cell (APC), and/or
5 - at least one second moiety is introduced which stimulates the immune system, and/or
- at least one third moiety is introduced which optimises presentation of the modified IL5 polypeptide to the immune system.

10

However, all these modifications should be carried out while maintaining a substantial fraction of the original B-lymphocyte epitopes in IL5, since the B-lymphocyte recognition of the native molecule is thereby enhanced.

15

In one preferred embodiment, side groups (in the form of foreign T-cell epitopes or the above-mentioned first, second and third moieties) are covalently or non-covalently introduced. This is intended to mean that stretches of amino acid residues
20 derived from IL5 are derivatized without altering the primary amino acid sequence, or at least without introducing changes in the peptide bonds between the individual amino acids in the chain.

25 An alternative, and preferred, embodiment utilises amino acid substitution and/or deletion and/or insertion and/or addition (which may be effected by recombinant means or by means of peptide synthesis; modifications which involves longer stretches of amino acids can give rise to fusion polypep-
30 tides). One especially preferred version of this embodiment is the technique described in WO 95/05849, which discloses a method for down-regulating self-proteins by immunising with analogues of the self-proteins wherein a number of amino acid sequence(s) has been substituted with a corresponding number
35 of amino acid sequence(s) which each comprise a foreign immunodominant T-cell epitope, while at the same time maintaining the overall tertiary structure of the self-protein in the analogue. For the purposes of the present invention, it is how-

ever sufficient if the modification (be it an amino acid in-
sertion, addition, deletion or substitution) gives rise to a
foreign T-cell epitope and at the same time preserves a sub-
stantial number of the B-cell epitopes in IL5. However, in or-
5 der to obtain maximum efficacy of the immune response induced,
it is preferred that the overall tertiary structure of IL5 is
maintained in the modified molecule.

The following formula describes the IL5 constructs generally
10 covered by the invention:

$$(MOD_1)_{s1}(IL5_{e1})_{n1}(MOD_2)_{s2}(IL5_{e2})_{n2}....(MOD_x)_{sx}(IL5_{ex})_{nx} \qquad (I)$$

-where $IL5_{e1}-IL5_{ex}$ are x B-cell epitope containing subsequences
15 of IL5 which independently are identical or non-identical and
which may contain or not contain foreign side groups, x is an
integer $\geq$ 3, n1-nx are x integers $\geq$ 0 (at least one is $\geq$ 1),
MOD_1-MOD_x are x modifications introduced between the preserved
B-cell epitopes, and s_1-s_x are x integers $\geq$ 0 (at least one is
20 $\geq$ 1 if no side groups are introduced in the $IL5_e$ sequences).
Thus, given the general functional restraints on the immuno-
genicity of the constructs, the invention allows for all kinds
of permutations of the original IL5 sequence, and all kinds of
modifications therein. Thus, included in the invention are
25 modified IL5 obtained by omission of parts of the IL5 sequence
which e.g. exhibit adverse effects *in vivo* or omission of
parts which could give rise to undesired immunological reac-
tions.

30 Maintenance of a substantial fraction of B-cell epitopes or
even the overall tertiary structure of a protein which is sub-
jected to modification as described herein can be achieved in
several ways. One is simply to prepare a polyclonal antiserum
directed against IL5 (e.g. an antiserum prepared in a rabbit)
35 and thereafter use this antiserum as a test reagent (e.g. in a
competitive ELISA) against the modified proteins which are
produced. Modified versions (analogues) which react to the
same extent with the antiserum as does IL5 must be regarded as

having the same overall tertiary structure as IL5 whereas ana-
logues exhibiting a limited (but still significant and spe-
cific) reactivity with such an antiserum are regarded as ha-
ving maintained a substantial fraction of the original B-cell
5 epitopes.

Alternatively, a selection of monoclonal antibodies reactive
with distinct epitopes on IL5 can be prepared and used as a
test panel. This approach has the advantage of allowing 1) an
10 epitope mapping of IL5 and 2) a mapping of the epitopes which
are maintained in the analogues prepared.

Of course, a third approach would be to resolve the 3-dimen-
sional structure of IL5 or of a biologically active truncate
15 thereof (cf. above) and compare this to the resolved three-di-
mensional structure of the analogues prepared. Three-dimen-
sional structure can be resolved by the aid of X-ray diffrac-
tion studies and NMR-spectroscopy. Further information rela-
ting to the tertiary structure can to some extent be obtained
20 from circular dichroism studies which have the advantage of
merely requiring the polypeptide in pure form (whereas X-ray
diffraction requires the provision of crystallized polypeptide
and NMR requires the provision of isotopic variants of the
polypeptide) in order to provide useful information about the
25 tertiary structure of a given molecule. However, ultimately X-
ray diffraction and/or NMR are necessary to obtain conclusive
data since circular dichroism can only provide indirect evi-
dence of correct 3-dimensional structure via information of
secondary structure elements.
30
One preferred embodiment of the invention utilises multiple
presentations of B-lymphocyte epitopes of IL5 (i.e. formula I
wherein at least one B-cell epitope is present in two posi-
tions). This effect can be achieved in various ways, e.g. by
35 simply preparing fusion polypeptides comprising the structure
$(IL5)_m$, where m is an integer ≥ 2 and then introduce the modi-
fications discussed herein in at least one of the IL5 se-
quences, or alternatively, inserted between at least two of

26

the IL5 amino acid sequences. It is preferred that the modifi-
cations introduced includes at least one duplication of a B-
lymphocyte epitope and/or the introduction of a hapten.

5   As mentioned above, the introduction of a foreign T-cell epi-
tope can be accomplished by introduction of at least one amino
acid insertion, addition, deletion, or substitution. Of
course, the normal situation will be the introduction of more
than one change in the amino acid sequence (e.g. insertion of
10 or substitution by a complete T-cell epitope) but the
important goal to reach is that the IL5 analogue, when
processed by an antigen presenting cell (APC), will give rise
to such a foreign immunodominant T-cell epitope being
presented in context of an MCH Class II molecule on the
15 surface of the APC. Thus, if the IL5 amino acid sequence in
appropriate positions comprises a number of amino acid
residues which can also be found in a foreign T_H epitope then
the introduction of a foreign T_H epitope can be accomplished by
providing the remaining amino acids of the foreign epitope by
20 means of amino acid insertion, addition, deletion and
substitution. In other words, it is not necessary to introduce
a complete T_H epitope by insertion or substitution.

It is preferred that the number of amino acid insertions, de-
25 letions, substitutions or additions is at least 2, such as 3,
4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,
and 25 insertions, substitutions, additions or deletions. It
is furthermore preferred that the number of amino acid inser-
tions, substitutions, additions or deletions is not in excess
30 of 150, such as at most 100, at most 90, at most 80, and at
most 70. It is especially preferred that the number of substi-
tutions, insertions, deletions, or additions does not exceed
60, and in particular the number should not exceed 50 or even
40. Most preferred is a number of not more than 30. With re-
35 spect to amino acid additions, it should be noted that these,
when the resulting construct is in the form of a fusion poly-
peptide, is often considerably higher than 150.

27

Preferred embodiments of the invention includes modification
by introducing at least one foreign immunodominant T_H epitope.
It will be understood that the question of immune dominance of
a T_H epitope depends on the animal species in question. As used
5 herein, the term "immunodominance" simply refers to epitopes
which in the vaccinated individual gives rise to a significant
immune response, but it is a well-known fact that a T_H epitope
which is immunodominant in one individual is not necessarily
immunodominant in another individual of the same species, even
10 though it may be capable of binding MHC-II molecules in the
latter individual.

Another important point is the issue of MHC restriction of T_H
epitopes. In general, naturally occurring T_H epitopes are MHC
15 restricted, i.e. a certain peptide constituting a T_H epitope
will only bind effectively to a subset of MHC Class II mole-
cules. This in turn has the effect that in most cases the use
of one specific T_H epitope will result in a vaccine component
which is effective in a fraction of the population only, and
20 depending on the size of that fraction, it can be necessary to
include more T_H epitopes in the same molecule, or alternatively
prepare a multi-component vaccine wherein the components are
IL5 variants which are distinguished from each other by the
nature of the T_H epitope introduced.
25
If the MHC restriction of the T-cells used is completely un-
known (for instance in a situation where the vaccinated animal
has a poorly defined MHC composition), the fraction of the
animal population covered by a specific vaccine composition
30 can be determined by means of the following formula:

$$f_{population} = 1 - \prod_{i=1}^{n} (1 - p_i) \tag{II}$$

-where p_i is the frequency in the population of responders to
35 the ith foreign T-cell epitope present in the vaccine composi-
tion, and n is the total number of foreign T-cell epitopes in
the vaccine composition. Thus, a vaccine composition contain-

ing 3 foreign T-cell epitopes having response frequencies in the population of 0.8, 0.7, and 0.6, respectively, would give

$$1 - 0.2 \times 0.3 \times 0.4 = 0.976$$

-*i.e.* 97.6 percent of the population will statistically mount an MHC-II mediated response to the vaccine.

The above formula does not apply in situations where a more or less precise MHC restriction pattern of the peptides used is known. If, for instance a certain peptide only binds the human MHC-II molecules encoded by HLA-DR alleles DR1, DR3, DR5, and DR7, then the use of this peptide together with another peptide which binds the remaining MHC-II molecules encoded by HLA-DR alleles will accomplish 100% coverage in the population in question. Likewise, if the second peptide only binds DR3 and DR5, the addition of this peptide will not increase the coverage at all. If one bases the calculation of population response purely on MHC restriction of T-cell epitopes in the vaccine, the fraction of the population covered by a specific vaccine composition can be determined by means of the following formula:

$$f_{population} = 1 - \prod_{j=1}^{3} (1 - \varphi_j)^2 \tag{III}$$

-wherein ϕ_j is the sum of frequencies in the population of allelic haplotypes encoding MHC molecules which bind any one of the T-cell epitopes in the vaccine and which belong to the jth of the 3 known HLA loci (DP, DR and DQ); in practice, it is first determined which MHC molecules will recognize each T-cell epitope in the vaccine and thereafter these MHC molecules are listed by type (DP, DR and DQ) – then, the individual frequencies of the different listed allelic haplotypes are summed for each type, thereby yielding ϕ_1, ϕ_2, and ϕ_3.

It may occur that the value p_i in formula II exceeds the corresponding theoretical value π_i:

$$\pi_i = 1 - \prod_{j=1}^{3}(1-v_j)^2 \qquad\qquad\qquad (IV)$$

-wherein v_j is the sum of frequencies in the population of allelic haplotypes encoding MHC molecules which bind the ith T-cell epitope in the vaccine and which belong to the jth of the 3 known HLA loci (DP, DR and DQ). This means that in $1-\pi_i$ of the population there is a frequency of responders of $f_{residual\_i} = (p_i-\pi_i)/(1-\pi_i)$. Therefore, formula III can be adjusted so as to yield formula V:

$$f_{population} = 1 - \prod_{j=1}^{3}(1-\varphi_j)^2 + \left(1-\prod_{i=1}^{n}(1-f_{residual\_i})\right) \qquad (V)$$

-where the term $1-f_{residual\_i}$ is set to zero if negative. It should be noted that formula V requires that all epitopes have been haplotype mapped against identical sets of haplotypes.

Therefore, when selecting T-cell epitopes to be introduced in the IL5 analogue, it is important to include all knowledge of the epitopes which is available: 1) The frequency of responders in the population to each epitope, 2) MHC restriction data, and 3) frequency in the population of the relevant haplotypes.

There exists a number of naturally occurring "promiscuous" T-cell epitopes which are active in a large proportion of individuals of an animal species or an animal population and these are preferably introduced in the vaccine, thereby reducing the need for a very large number of different IL5 analogues in the same vaccine.

The promiscuous epitope can according to the invention be a naturally occurring human T-cell epitope such as epitopes from tetanus toxoid (e.g. the P2 and P30 epitopes), diphtheria toxoid, Influenza virus hemagluttinin (HA), and *P. falciparum* CS antigen.

Over the years a number of other promiscuous T-cell epitopes have been identified. Especially peptides capable of binding a

large proportion of HLA-DR molecules encoded by the different
HLA-DR alleles have been identified and these are all possible
T-cell epitopes to be introduced in the IL5 analogues used ac-
cording to the present invention. Cf. also the epitopes dis-
5   cussed in the following references which are hereby all incor-
porated by reference herein: WO 98/23635 (Frazer IH et al.,
assigned to The University of Queensland); Southwood S et. al,
1998, J. Immunol. 160: 3363-3373; Sinigaglia F et al., 1988,
Nature 336: 778-780; Chicz RM et al., 1993, J. Exp. Med 178:
10 27-47; Hammer J et al., 1993, Cell 74: 197-203; and Falk K et
al., 1994, Immunogenetics 39: 230-242. The latter reference
also deals with HLA-DQ and -DP ligands. All epitopes listed in
these 5 references are relevant as candidate natural epitopes
to be used in the present invention, as are epitopes which
15 share common motifs with these.

Alternatively, the epitope can be any artificial T-cell epi-
tope which is capable of binding a large proportion of MHC
Class II molecules. In this context the pan DR epitope
20 peptides ("PADRE") described in WO 95/07707 and in the
corresponding paper Alexander J et al., 1994, Immunity 1: 751-
761 (both disclosures are incorporated by reference herein)
are interesting candidates for epitopes to be used according
to the present invention. It should be noted that the most
25 effective PADRE peptides disclosed in these papers carry D-
amino acids in the C- and N-termini in order to improve
stability when administered. However, the present invention
primarily aims at incorporating the relevant epitopes as part
of the modified IL5 which should then subsequently be broken
30 down enzymatically inside the lysosomal compartment of APCs to
allow subsequent presentation in the context of an MHC-II
molecule and therefore it is not expedient to incorporate D-
amino acids in the epitopes used in the present invention.

35 One especially preferred PADRE peptide is the one having the
amino acid sequence AKFVAAWTLKAAA (SEQ ID NO: 65) or an immu-
nologically effective subsequence thereof. This, and other
epitopes having the same lack of MHC restriction are preferred

T-cell epitopes which should be present in the IL5 analogues
used in the inventive method. Such super-promiscuous epitopes
will allow for the most simple embodiments of the invention
wherein only one single modified IL5 is presented to the vac-
5 cinated animal's immune system.

As mentioned above, the modification of IL5 can also include
the introduction of a first moiety which targets the modified
IL5 to an APC or a B-lymphocyte. For instance, the first mo-
10 iety can be a specific binding partner for a B-lymphocyte spe-
cific surface antigen or for an APC specific surface antigen.
Many such specific surface antigens are known in the art. For
instance, the moiety can be a carbohydrate for which there is
a receptor on the B-lymphocyte or on the APC (e.g. mannan or
15 mannose). Alternatively, the second moiety can be a hapten.
Also an antibody fragment which specifically recognizes a sur-
face molecule on APCs or lymphocytes can be used as a first
moiety (the surface molecule can e.g. be an FCγ receptor of
macrophages and monocytes, such as FCγRI or, alternatively any
20 other specific surface marker such as CD40 or CTLA-4). It
should be noted that all these exemplary targeting molecules
can be used as part of an adjuvant also, cf. below.

As an alternative or supplement to targeting the modified IL5
25 polypeptide to a certain cell type in order to achieve an en-
hanced immune response, it is possible to increase the level
of responsiveness of the immune system by including the above-
mentioned second moiety which stimulates the immune system.
Typical examples of such second moieties are cytokines, and
30 heat-shock proteins or molecular chaperones, as well as effec-
tive parts thereof.

Suitable cytokines to be used according to the invention are
those which will normally also function as adjuvants in a vac-
35 cine composition, i.e. for instance interferon γ (IFN-γ),
Flt3L, interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin
4 (IL-4), interleukin 6 (IL-6), interleukin 12 (IL-12), inter-
leukin 13 (IL-13), interleukin 15 (IL-15), and granulocyte-

macrophage colony stimulating factor (GM-CSF); alternatively, the functional part of the cytokine molecule may suffice as the second moiety. With respect to the use of such cytokines as adjuvant substances, cf. the discussion below. It should be 5 noted that use of both IL-4 and IL-13 should be exercised very carefully, if at all, as both molecules are known as key effector molecules in the pathophysiology of atopy and asthma.

According to the invention, suitable heat-shock proteins or 10 molecular chaperones used as the second moiety can be HSP70, HSP90, HSC70, GRP94 (also known as gp96, cf. Wearsch PA et al. 1998, Biochemistry **37**: 5709-19), and CRT (calreticulin).

Alternatively, the second moiety can be a toxin, such as li-15 steriolycin (LLO), lipid A and heat-labile enterotoxin. Also, a number of mycobacterial derivatives such as MDP (muramyl dipeptide) and the trehalose diesters TDM and TDE are interesting possibilities.

20 Also the possibility of introducing a third moiety which enhances the presentation of the modified IL5 to the immune system is an important embodiment of the invention. The art has shown several examples of this principle. For instance, it is known that the palmitoyl lipidation anchor in the *Borrelia* 25 *burgdorferi* protein OspA can be utilised so as to provide self-adjuvating polypeptides (cf. e.g. WO 96/40718). It seems that the lipidated proteins form up micelle-like structures with a core consisting of the lipidation anchor parts of the polypeptides and the remaining parts of the molecule protru-30 ding therefrom, resulting in multiple presentations of the antigenic determinants. Hence, the use of this and related approaches using different lipidation anchors (e.g. a myristyl group, a myristyl group, a farnesyl group, a geranyl-geranyl group, a GPI-anchor, and an N-acyl diglyceride group) are pre-35 ferred embodiments of the invention, especially since the provision of such a lipidation anchor in a recombinantly produced protein is fairly straightforward and merely requires use of e.g. a naturally occurring signal sequence as a fusion partner

33

for the modified IL5 polypeptide. Another possibility is use
of the C3d fragment of complement factor C3 or C3 itself (cf.
Dempsey et al., 1996, Science 271, 348-350 and Lou & Kohler,
1998, Nature Biotechnology 16, 458-462).

5

An alternative embodiment of the invention which also results
in the preferred presentation of multiple (e.g. at least 2)
copies of the important epitopic regions of IL5 to the immune
system is the covalent or non-covalent coupling of IL5, subse-
10 quence or variants thereof to certain carrier molecules. For
instance, polymers can be used, e.g. carbohydrates such as
dextran, cf. e.g. Lees A et al., 1994, Vaccine 12: 1160-1166;
Lees A et al., 1990, J Immunol. 145: 3594-3600, but also man-
nose and mannan are useful alternatives. Integral membrane
15 proteins from e.g. E. coli and other bacteria are also useful
conjugation partners. The traditional carrier molecules such
as keyhole limpet hemocyanin (KLH), tetanus toxoid, diphtheria
toxoid, and bovine serum albumin (BSA) are also preferred and
useful conjugation partners.
20

Certain areas of native IL5 are believed to be superiorly
suited for performing modifications. It is predicted that
modifications in at least one of loops 1-3 or in the amino
acid residues C-terminal to helix D (said loops and said helix
25 D corresponding to those shown in Fig. 3 for human and murine
IL5) will be most likely to produce the desired constructs and
vaccination results. Considerations underlying these chosen
areas are a) preservation of known and predicted B-cell epi-
topes, b) preservation of tertiary and quaternary structures
30 etc, cf. also the discussion in the preamble to the examples.
At any rate, as discussed above, it is fairly easy to screen a
set of modified IL5 molecules which have all been subjected to
introduction of a T-cell epitope in different locations.

35 Since the most preferred embodiments of the present invention
involves down-regulation of human IL5, it is consequently pre-
ferred that the IL5 polypeptide discussed above is a human IL5
polypeptide. In this embodiment, it is especially preferred

that the human IL5 polypeptide has been modified by substitu-
ting at least one amino acid sequence in SEQ ID NO: 1 with at
least one amino acid sequence of equal or different length and
containing a foreign T_H epitope, wherein substituted amino acid
5 residues are selected from the group consisting of residues
87-90, residues 32-43, residues 59-64, residues 86-91, and
residues 110-113. The rationale behind such constructs is dis-
cussed in detail in the examples.

10 Formulation of IL5 and modified IL5 polypeptides

When effecting presentation of the IL5 polypeptide or the
modified IL5 polypeptide to an animal's immune system by means
of administration thereof to the animal, the formulation of
15 the polypeptide follows the principles generally acknowledged
in the art.

Preparation of vaccines which contain peptide sequences as ac-
tive ingredients is generally well understood in the art, as
20 exemplified by U.S. Patents 4,608,251; 4,601,903; 4,599,231;
4,599,230; 4,596,792; and 4,578,770, all incorporated herein
by reference. Typically, such vaccines are prepared as in-
jectables either as liquid solutions or suspensions; solid
forms suitable for solution in, or suspension in, liquid prior
25 to injection may also be prepared. The preparation may also be
emulsified. The active immunogenic ingredient is often mixed
with excipients which are pharmaceutically acceptable and com-
patible with the active ingredient. Suitable excipients are,
for example, water, saline, dextrose, glycerol, ethanol, or
30 the like, and combinations thereof. In addition, if desired,
the vaccine may contain minor amounts of auxiliary substances
such as wetting or emulsifying agents, pH buffering agents, or
adjuvants which enhance the effectiveness of the vaccines; cf.
the detailed discussion of adjuvants below.
35
The vaccines are conventionally administered parenterally, by
injection, for example, either subcutaneously, intracutane-
ously, intradermally, subdermally or intramuscularly. Addi-

tional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral, buccal, sublinqual, intraperitoneal, intravaginal, anal, epidural, spinal, and intracranial formulations. For supposito-
5 ries, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for exam-
10 ple, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10-95% of active in-
15 gredient, preferably 25-70%. For oral formulations, cholera toxin is an interesting formulation partner (and also a possible conjugation partner).

The polypeptides may be formulated into the vaccine as neutral
20 or salt forms. Pharmaceutically acceptable salts include acid addition salts (formed with the free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like.
25 Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.
30
The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, including, e.g.,
35 the capacity of the individual's immune system to mount an immune response, and the degree of protection desired. Suitable dosage ranges are of the order of several hundred micrograms active ingredient per vaccination with a preferred range from

about 0.1 µg to 2,000 µg (even though higher amounts in the 1-
10 mg range are contemplated), such as in the range from about
0.5 µg to 1,000 µg, preferably in the range from 1 µg to 500
µg and especially in the range from about 10 µg to 100 µg.
5 Suitable regimens for initial administration and booster shots
are also variable but are typified by an initial administra-
tion followed by subsequent inoculations or other administra-
tions.

10 The manner of application may be varied widely. Any of the
conventional methods for administration of a vaccine are ap-
plicable. These include oral application on a solid physio-
logically acceptable base or in a physiologically acceptable
dispersion, parenterally, by injection or the like. The dosage
15 of the vaccine will depend on the route of administration and
will vary according to the age of the person to be vaccinated
and the formulation of the antigen.

Some of the polypeptides of the vaccine are sufficiently immu-
20 nogenic in a vaccine, but for some of the others the immune
response will be enhanced if the vaccine further comprises an
adjuvant substance.

Various methods of achieving adjuvant effect for the vaccine
25 are known. General principles and methods are detailed in "The
Theory and Practical Application of Adjuvants", 1995, Duncan
E.S. Stewart-Tull (ed.), John Wiley & Sons Ltd, ISBN 0-471-
95170-6, and also in "Vaccines: New Generation Immunological
Adjuvants", 1995, Gregoriadis G et al. (eds.), Plenum Press,
30 New York, ISBN 0-306-45283-9, both of which are hereby incor-
porated by reference herein.

It is especially preferred to use an adjuvant which can be
demonstrated to facilitate breaking of the autotolerance to
35 autoantigens; in fact, this is essential in cases where un-
modified IL5 is used as the active ingredient in the autovac-
cine. Non-limiting examples of suitable adjuvants are selected
from the group consisting of an immune targeting adjuvant; an

immune modulating adjuvant such as a toxin, a cytokine, and a
mycobacterial derivative; an oil formulation; a polymer; a mi-
celle forming adjuvant; a saponin; an immunostimulating com-
plex matrix (ISCOM matrix); a particle; DDA; aluminium adju-
5 vants; DNA adjuvants; γ-inulin; and an encapsulating adjuvant.
In general it should be noted that the disclosures above which
relate to compounds and agents useful as first, second and
third moieties in the analogues also refer *mutatis mutandis* to
their use in the adjuvant of a vaccine of the invention.

10

The application of adjuvants include use of agents such as
aluminium hydroxide or phosphate (alum), commonly used as 0.05
to 0.1 percent solution in buffered saline, admixture with
synthetic polymers of sugars (e.g. Carbopol®) used as 0.25
15 percent solution, aggregation of the protein in the vaccine by
heat treatment with temperatures ranging between 70° to 101°C
for 30 second to 2 minute periods respectively and also aggre-
gation by means of cross-linking agents are possible. Aggrega-
tion by reactivation with pepsin treated antibodies (Fab frag-
20 ments) to albumin, mixture with bacterial cells such as *C.
parvum* or endotoxins or lipopolysaccharide components of gram-
negative bacteria, emulsion in physiologically acceptable oil
vehicles such as mannide mono-oleate (Aracel A) or emulsion
with 20 percent solution of a perfluorocarbon (Fluosol-DA)
25 used as a block substitute may also be employed. Admixture
with oils such as squalene and IFA is also preferred.

According to the invention DDA (dimethyldioctadecylammonium
bromide) is an interesting candidate for an adjuvant as is DNA
30 and γ-inulin, but also Freund's complete and incomplete adju-
vants as well as *quillaja* saponins such as QuilA and QS21 are
interesting as is RIBI. Further possibilities are monophos-
phoryl lipid A (MPL), the above mentioned C3 and C3d, and mu-
ramyl dipeptide (MDP).

35

Liposome formulations are also known to confer adjuvant ef-
fects, and therefore liposome adjuvants are preferred accor-
ding to the invention.

38

Also immunostimulating complex matrix type (ISCOM® matrix) ad-
juvants are preferred choices according to the invention, es-
pecially since it has been shown that this type of adjuvants
5 are capable of up-regulating MHC Class II expression by APCs.
An ISCOM® matrix consists of (optionally fractionated) sapo-
nins (triterpenoids) from *Quillaja saponaria*, cholesterol, and
phospholipid. When admixed with the immunogenic protein, the
resulting particulate formulation is what is known as an ISCOM
10 particle where the saponin constitutes 60-70% w/w, the choles-
terol and phospholipid 10-15% w/w, and the protein 10-15% w/w.
Details relating to composition and use of immunostimulating
complexes can e.g. be found in the above-mentioned text-books
dealing with adjuvants, but also Morein B *et al.*, 1995, Clin.
15 Immunother. **3**: 461-475 as well as Barr IG and Mitchell GF,
1996, Immunol. and Cell Biol. **74**: 8-25 (both incorporated by
reference herein) provide useful instructions for the prepara-
tion of complete immunostimulating complexes.

20 Another highly interesting (and thus, preferred) possibility
of achieving adjuvant effect is to employ the technique de-
scribed in Gosselin et al., 1992 (which is hereby incorporated
by reference herein). In brief, the presentation of a relevant
antigen such as an antigen of the present invention can be en-
25 hanced by conjugating the antigen to antibodies (or antigen
binding antibody fragments) against the Fcγ receptors on mono-
cytes/macrophages. Especially conjugates between antigen and
anti-FcγRI have been demonstrated to enhance immunogenicity for
the purposes of vaccination.
30
Other possibilities involve the use of the targeting and im-
mune modulating substances (*i.a.* cytokines) mentioned above as
candidates for the first and second moieties in the modified
versions of IL5. In this connection, also synthetic inducers
35 of cytokines like poly I:C are possibilities.

Suitable mycobacterial derivatives are selected from the group
consisting of muramyl dipeptide, complete Freund's adjuvant,
RIBI, and a diester of trehalose such as TDM and TDE.

5   Suitable immune targeting adjuvants are selected from the
group consisting of CD40 ligand and CD40 antibodies or spe-
cifically binding fragments thereof (cf. the discussion
above), mannose, a Fab fragment, and CTLA-4.

10 Suitable polymer adjuvants are selected from the group con-
sisting of a carbohydrate such as dextran, PEG, starch, man-
nan, and mannose; a plastic polymer such as; and latex such as
latex beads.

15 Yet another interesting way of modulating an immune response
is to include the IL5 immunogen (optionally together with ad-
juvants and pharmaceutically acceptable carriers and vehicles)
in a "virtual lymph node" (VLN) (a proprietary medical device
developed by ImmunoTherapy, Inc., 360 Lexington Avenue, New
20 York, NY 10017-6501). The VLN (a thin tubular device) mimics
the structure and function of a lymph node. Insertion of a VLN
under the skin creates a site of sterile inflammation with an
upsurge of cytokines and chemokines. T- and B-cells as well as
APCs rapidly respond to the danger signals, home to the in-
25 flamed site and accumulate inside the porous matrix of the
VLN. It has been shown that the necessary antigen dose re-
quired to mount an immune response to an antigen is reduced
when using the VLN and that immune protection conferred by
vaccination using a VLN surpassed conventional immunization
30 using Ribi as an adjuvant. The technology is *i.a.* described
briefly in Gelber C *et al.*, 1998, "Elicitation of Robust Cel-
lular and Humoral Immune Responses to Small Amounts of Immuno-
gens Using a Novel Medical Device Designated the Virtual Lymph
Node", in: "From the Laboratory to the Clinic, Book of Ab-
35 stracts, October 12th – 15th 1998, Seascape Resort, Aptos, Cali-
fornia".

40

It is expected that the vaccine should be administered at
least once a year, such as at least 1, 2, 3, 4, 5, 6, and 12
times a year. More specifically, 1-12 times per year is ex-
pected, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 times
5   a year to an individual in need thereof. It has previously
been shown that the memory immunity induced by the use of the
preferred autovaccines according to the invention is not per-
manent, and therefor the immune system needs to be periodi-
cally challenged with the analogues.
10

Due to genetic variation, different individuals may react with
immune responses of varying strength to the same polypeptide.
Therefore, the vaccine according to the invention may comprise
several different polypeptides in order to increase the immune
15 response, cf. also the discussion above concerning the choice
of foreign T-cell epitope introductions. The vaccine may com-
prise two or more polypeptides, where all of the polypeptides
are as defined above.

20 The vaccine may consequently comprise 3-20 different modified
or unmodified polypeptides, such as 3-10 different polypep-
tides. However, normally the number of polypeptides will be
sought kept to a minimum such as 1 or 2 polypeptides.

25 Nucleic acid vaccination

As an alternative to classic administration of a peptide-based
vaccine, the technology of nucleic acid vaccination (also
known as "nucleic acid immunisation", "genetic immunisation",
30 and "gene immunisation") offers a number of attractive fea-
tures.

First, in contrast to the traditional vaccine approach, nu-
cleic acid vaccination does not require resource consuming
35 large-scale production of the immunogenic agent (e.g. in the
form of industrial scale fermentation of microorganisms pro-
ducing modified IL5). Furthermore, there is no need to device
purification and refolding schemes for the immunogen. And fi-

41

nally, since nucleic acid vaccination relies on the biochemical apparatus of the vaccinated individual in order to produce the expression product of the nucleic acid introduced, the optimum posttranslational processing of the expression

5 product is expected to occur; this is especially important in the case of autovaccination, since, as mentioned above, a significant fraction of the original IL5 B-cell epitopes should be preserved in the modified molecule, and since B-cell epitopes in principle can be constituted by parts of any

10 (bio)molecule (e.g. carbohydrate, lipid, protein etc.). Therefore, native glycosylation and lipidation patterns of the immunogen may very well be of importance for the overall immunogenicity and this is expected to be ensured by having the host producing the immunogen.

15

Hence, a preferred embodiment of the invention comprises effecting presentation of modified IL5 to the immune system by introducing nucleic acid(s) encoding the modified IL5 into the animal's cells and thereby obtaining *in vivo* expression by the

20 cells of the nucleic acid(s) introduced.

In this embodiment, the introduced nucleic acid is preferably DNA which can be in the form of naked DNA, DNA formulated with charged or uncharged lipids, DNA formulated in liposomes, DNA

25 included in a viral vector, DNA formulated with a transfection-facilitating protein or polypeptide, DNA formulated with a targeting protein or polypeptide, DNA formulated with Calcium precipitating agents, DNA coupled to an inert carrier molecule, DNA encapsulated in a polymer, e.g. in PLGA (cf. the

30 microencapsulation technology described in WO 98/31398) or in chitin or chitosan, and DNA formulated with an adjuvant. In this context it is noted that practically all considerations pertaining to the use of adjuvants in traditional vaccine formulation apply for the formulation of DNA vaccines. Hence, all

35 disclosures herein which relate to use of adjuvants in the context of polypeptide based vaccines apply *mutatis mutandis* to their use in nucleic acid vaccination technology.

42

As for routes of administration and administration schemes of polypeptide based vaccines which have been detailed above, these are also applicable for the nucleic acid vaccines of the invention and all discussions above pertaining to routes of

5 administration and administration schemes for polypeptides apply *mutatis mutandis* to nucleic acids. To this should be added that nucleic acid vaccines can suitably be administered intraveneously and intraarterially. Furthermore, it is well-known in the art that nucleic acid vaccines can be administered by

10 use of a so-called gene gun, and hence also this and equivalent modes of administration are regarded as part of the present invention. Finally, also the use of a VLN in the administration of nucleic acids has been reported to yield good results, and therefore this particular mode of administration is

15 particularly preferred.

Furthermore, the nucleic acid(s) used as an immunization agent can contain regions encoding the 1st, 2nd and/or 3rd moieties, e.g. in the form of the immunomodulating substances described

20 above such as the cytokines discussed as useful adjuvants. A preferred version of this embodiment encompasses having the coding region for the analogue and the coding region for the immunomodulator in different reading frames or at least under the control of different promoters. Thereby it is avoided that

25 the analogue or epitope is produced as a fusion partner to the immunomodulator. Alternatively, two distinct nucleotide fragments can be used, but this is less preferred because of the advantage of ensured co-expression when having both coding regions included in the same molecule.

30

Accordingly, the invention also relates to a composition for inducing production of antibodies against IL5, the composition comprising

-       a nucleic acid fragment or a vector of the invention (cf.

35   the discussion of vectors below), and

-       a pharmaceutically and immunologically acceptable vehicle
        and/or carrier and/or adjuvant as discussed above.

Under normal circumstances, the IL5 variant-encoding nucleic
acid is introduced in the form of a vector wherein expression
is under control of a viral promoter. For more detailed dis-
cussions of vectors and DNA fragments according to the inven-
5 tion, cf. the discussion below. Also, detailed disclosures re-
lating to the formulation and use of nucleic acid vaccines are
available, cf. Donnelly JJ et al, 1997, Annu. Rev. Immunol.
15: 617-648 and Donnelly JJ et al., 1997, Life Sciences 60:
163-172. Both of these references are incorporated by refe-
10 rence herein.

Live vaccines

A third alternative for effecting presentation of modified IL5
15 to the immune system is the use of live vaccine technology. In
live vaccination, presentation to the immune system is ef-
fected by administering, to the animal, a non-pathogenic mi-
croorganism which has been transformed with a nucleic acid
fragment encoding a modified IL5 or with a vector incorpora-
20 ting such a nucleic acid fragment. The non-pathogenic microor-
ganism can be any suitable attenuated bacterial strain (at-
tenuated by means of passaging or by means of removal of
pathogenic expression products by recombinant DNA technology),
e.g. Mycobacterium bovis BCG., non-pathogenic Streptococcus
25 spp., E. coli, Salmonella spp., Vibrio cholerae, Shigella,
etc. Reviews dealing with preparation of state-of-the-art live
vaccines can e.g. be found in Saliou P, 1995, Rev. Prat. 45:
1492-1496 and Walker PD, 1992, Vaccine 10: 977-990, both in-
corporated by reference herein. For details about the nucleic
30 acid fragments and vectors used in such live vaccines, cf. the
discussion below.

As an alternative to bacterial live vaccines, the nucleic acid
fragment of the invention discussed below can be incorporated
35 in a non-virulent viral vaccine vector such as a vaccinia
strain or any other suitable pox virus.

44

Normally, the non-pathogenic microorganism or virus is admi-
nistered only once to the animal, but in certain cases it may
be necessary to administer the microorganism more than once in
a lifetime in order to maintain protective immunity. It is
5 even contemplated that immunization schemes as those detailed
above for polypeptide vaccination will be useful when using
live or virus vaccines.

Alternatively, live or virus vaccination is combined with pre-
10 vious or subsequent polypeptide and/or nucleic acid vaccina-
tion. For instance, it is possible to effect primary immuniza-
tion with a live or virus vaccine followed by subsequent
booster immunizations using the polypeptide or nucleic acid
approach.
15

The microorganism or virus can be transformed with nucleic
acid(s) containing regions encoding the 1st, 2nd and/or 3rd
moieties, e.g. in the form of the immunomodulating substances
described above such as the cytokines discussed as useful ad-
20 juvants. A preferred version of this embodiment encompasses
having the coding region for the analogue and the coding re-
gion for the immunomodulator in different reading frames or at
least under the control of different promoters. Thereby it is
avoided that the analogue or epitopes are produced as fusion
25 partners to the immunomodulator. Alternatively, two distinct
nucleotide fragments can be used as transforming agents. Of
course, having the 1st and/or 2nd and/or 3rd moieties in the
same reading frame can provide as an expression product, an
analogue of the invention, and such an embodiment is espe-
30 cially preferred according to the present invention.

Use of the method of the invention in disease treatment

As will be appreciated from the discussions above, the provi-
35 sion of the method of the invention allows for control of dis-
eases characterized by eosinophilia. In this context, asthma
is the key target for the inventive method but also other
chronic allergic conditions such as multiple allergy and al-

lergic rhinitis are feasible targets for treat-
ment/amelioration. Hence, an important embodiment of the
method of the invention for down-regulating IL5 activity com-
prises treating and/or preventing and/or ameliorating asthma
5 or other chronic allergic conditions characterized by eosino-
philia, the method comprising down-regulating IL5 activity ac-
cording to the method of the invention to such an extent that
the number of eosinophil cells is significantly reduced.

10 In the present context such a significant reduction in eosino-
phil cell numbers is at least 20% compared to the eosinophil
number prior to treatment, but higher percentages are contem-
plated, such as at least 30%, at least 40%, at least 50%, at
least 60%, at least 70%, at least 80% and even at least 90%.
15 The reduction may be systemic or, more often, locally in e.g.
the lungs.

Eosinophil cell numbers are determined by methods known in the
art, typically using microscopy of a suitable sample (such as
20 a BAL fluid) and counting the number of eosinophil cells manu-
ally under microscope. Alternatively, eosinophil numbers can
be counted using flow cytometric methods or any other conven-
ient method of cytometry capable of distinguishing eosino-
phils.
25
Peptides, polypeptides, and compositions of the invention

As will be apparent from the above, the present invention is
based on the concept of immunising individuals against the IL5
30 antigen in order to indirectly obtain a reduction in eosino-
phil cell numbers. The preferred way of obtaining such an im-
munization is to use modified versions of IL5, thereby provi-
ding molecules which have not previously been disclosed in the
art.
35
It is believed that the modified IL5 molecules discussed
herein are inventive in their own right, and therefore an im-
portant part of the invention pertains to an IL5 analogue

46

which is derived from an animal IL5 wherein is introduced a
modification which has as a result that immunization of the
animal with the analogue induces production of antibodies
cross-reacting with the unmodified IL5 polypeptide. Prefer-
5 ably, the nature of the modification conforms with the types
of modifications described above when discussing various em-
bodiments of the method of the invention when using modified
IL5. Hence, any disclosure presented herein pertaining to
modified IL5 molecules are relevant for the purpose of de-
10 scribing the IL5 analogues of the invention, and any such dis-
closures apply *mutatis mutandis* to the description of these
analogues.

It should be noted that preferred modified IL5 molecules com-
15 prise modifications which results in a polypeptide having a
sequence identity of at least 70% with IL5 or with a subse-
quence thereof of at least 10 amino acids in length. Higher
sequence identities are preferred, e.g. at least 75% or even
at least 80% or 85%. The sequence identity for proteins and
20 nucleic acids can be calculated as $(N_{ref} - N_{dif}) \cdot 100/N_{ref}$,
wherein N_{dif} is the total number of non-identical residues in
the two sequences when aligned and wherein N_{ref} is the number
of residues in one of the sequences. Hence, the DNA sequence
AGTCAGTC will have a sequence identity of 75% with the se-
25 quence AATCAATC (N_{dif}=2 and N_{ref}=8).

The invention also pertains to compositions useful in exerci-
sing the method of the invention. Hence, the invention also
relates to an immunogenic composition comprising an immuno-
30 genically effective amount of an IL5 polypeptide which is a
self-protein in an animal, said IL5 polypeptide being formu-
lated together with an immunologically acceptable adjuvant so
as to break the animal's autotolerance towards the IL5 poly-
peptide, the composition further comprising a pharmaceutically
35 and immunologically acceptable vehicle and/or carrier. In
other words, this part of the invention pertains to the formu-
lations of naturally occurring IL5 polypeptides which have

47

been described in connection with embodiments of the method of
the invention.

The invention also relates to an immunogenic composition com-
5 prising an immunologically effective amount of an IL5 analogue
defined above, said composition further comprising a pharma-
ceutically and immunologically acceptable diluent and/or ve-
hicle and/or carrier and/or excipient and optionally an adju-
vant. In other words, this part of the invention concerns for-
10 mulations of modified IL5, essentially as described herein-
above. The choice of adjuvants, carriers, and vehicles is ac-
cordingly in line with what has been discussed above when re-
ferring to formulation of modified and unmodified IL5 for use
in the inventive method for the down-regulation of IL5.

15

The polypeptides are prepared according to methods well-known
in the art. Longer polypeptides are normally prepared by means
of recombinant gene technology including introduction of a nu-
cleic acid sequence encoding the IL5 analogue into a suitable
20 vector, transformation of a suitable host cell with the vec-
tor, expression of the nucleic acid sequence, recovery of the
expression product from the host cells or their culture super-
natant, and subsequent purification and optional further modi-
fication, e.g. refolding or derivatization.

25

Shorter peptides are preferably prepared by means of the well-
known techniques of solid- or liquid-phase peptide synthesis.
However, recent advances in this technology has rendered pos-
sible the production of full-length polypeptides and proteins
30 by these means, and therefore it is also within the scope of
the present invention to prepare the long constructs by syn-
thetic means.

Nucleic acid fragments and vectors of the invention

35

It will be appreciated from the above disclosure that modified
IL5 polypeptides can be prepared by means of recombinant gene
technology but also by means of chemical synthesis or semisyn-

48

thesis; the latter two options are especially relevant when
the modification consists in coupling to protein carriers
(such as KLH, diphtheria toxoid, tetanus toxoid, and BSA) and
non-proteinaceous molecules such as carbohydrate polymers and
5 of course also when the modification comprises addition of
side chains or side groups to an IL5 polypeptide-derived pep-
tide chain.

For the purpose of recombinant gene technology, and of course
10 also for the purpose of nucleic acid immunization, nucleic
acid fragments encoding modified IL5 are important chemical
products. Hence, an important part of the invention pertains
to a nucleic acid fragment which encodes an IL5 analogue, *i.e.*
an IL5 derived polypeptide which either comprises the natural
15 IL5 sequence to which has been added or inserted a fusion
partner or, preferably an IL5 derived polypeptide wherein has
been introduced a foreign T-cell epitope by means of insertion
and/or addition, preferably by means of substitution and/or
deletion. The nucleic acid fragments of the invention are ei-
20 ther DNA or RNA fragments.

The nucleic acid fragments of the invention will normally be
inserted in suitable vectors to form cloning or expression
vectors carrying the nucleic acid fragments of the invention;
25 such novel vectors are also part of the invention. Details
concerning the construction of these vectors of the invention
will be discussed in context of transformed cells and microor-
ganisms below. The vectors can, depending on purpose and type
of application, be in the form of plasmids, phages, cosmids,
30 mini-chromosomes, or virus, but also naked DNA which is only
expressed transiently in certain cells is an important vector.
Preferred cloning and expression vectors of the invention are
capable of autonomous replication, thereby enabling high copy-
numbers for the purposes of high-level expression or high-
35 level replication for subsequent cloning.

The general outline of a vector of the invention comprises the
following features in the 5'→3' direction and in operable

linkage: a promoter for driving expression of the nucleic acid fragment of the invention, optionally a nucleic acid sequence encoding a leader peptide enabling secretion (to the extracellular phase or, where applicable, into the periplasma) of or

5   integration into the membrane of the polypeptide fragment, the nucleic acid fragment of the invention, and optionally a nucleic acid sequence encoding a terminator. When operating with expression vectors in producer strains or cell-lines it is for the purposes of genetic stability of the transformed cell pre-

10   ferred that the vector when introduced into a host cell is integrated in the host cell genome. In contrast, when working with vectors to be used for effecting *in vivo* expression in an animal (*i.e.* when using the vector in DNA vaccination) it is for security reasons preferred that the vector is not incapa-

15   ble of being integrated in the host cell genome; typically, naked DNA or non-integrating viral vectors are used, the choices of which are well-known to the person skilled in the art.

20   The vectors of the invention are used to transform host cells to produce the modified IL5 polypeptide of the invention. Such transformed cells, which are also part of the invention, can be cultured cells or cell lines used for propagation of the nucleic acid fragments and vectors of the invention, or used

25   for recombinant production of the modified IL5 polypeptides of the invention. Alternatively, the transformed cells can be suitable live vaccine strains wherein the nucleic acid fragment (one single or multiple copies) have been inserted so as to effect secretion or integration into the bacterial membrane

30   or cell-wall of the modified IL5.

Preferred transformed cells of the invention are microorganisms such as bacteria (such as the species *Escherichia* [e.g. *E. coli*], *Bacillus* [e.g. *Bacillus subtilis*], *Salmonella*, or

35   *Mycobacterium* [preferably non-pathogenic, e.g. *M. bovis* BCG]), yeasts (such as *Saccharomyces cerevisiae*), and protozoans. Alternatively, the transformed cells are derived from a multi-cellular organism such as a fungus, an insect cell, a plant

50

cell, or a mammalian cell. Most preferred are cells derived
from a human being, cf. the discussion of cell lines and vec-
tors below. Recent results have shown great promise in the use
of a commercially available *Drosophila melanogaster* cell line

5 (the Schneider 2 (S$_2$)cell line and vector system available from
Invitrogen) for the recombinant production of IL5 analogues of
the invention, and therefore this expression system is par-
ticularly preferred.

10 For the purposes of cloning and/or optimized expression it is
preferred that the transformed cell is capable of replicating
the nucleic acid fragment of the invention. Cells expressing
the nucleic fragment are preferred useful embodiments of the
invention; they can be used for small-scale or large-scale

15 preparation of the modified IL5 or, in the case of non-patho-
genic bacteria, as vaccine constituents in a live vaccine.

When producing the modified IL5 of the invention by means of
transformed cells, it is convenient, although far from essen-

20 tial, that the expression product is either exported out into
the culture medium or carried on the surface of the trans-
formed cell.

When an effective producer cell has been identified it is pre-

25 ferred, on the basis thereof, to establish a stable cell line
which carries the vector of the invention and which expresses
the nucleic acid fragment encoding the modified IL5. Prefer-
ably, this stable cell line secretes or carries the IL5 ana-
logue of the invention, thereby facilitating purification

30 thereof.

In general, plasmid vectors containing replicon and control
sequences which are derived from species compatible with the
host cell are used in connection with the hosts. The vector

35 ordinarily carries a replication site, as well as marking se-
quences which are capable of providing phenotypic selection in
transformed cells. For example, *E. coli* is typically trans-
formed using pBR322, a plasmid derived from an *E. coli* species

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(see, e.g., Bolivar et al., 1977). The pBR322 plasmid contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR plasmid, or other microbial plasmid or phage must also contain, or be modified to contain, promoters which can be used by the prokaryotic microorganism for expression.

Those promoters most commonly used in prokaryotic recombinant DNA construction include the B-lactamase (penicillinase) and
10 lactose promoter systems (Chang et al., 1978; Itakura et al., 1977; Goeddel et al., 1979) and a tryptophan (trp) promoter system (Goeddel et al., 1979; EP-A-0 036 776). While these are the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleo-
15 tide sequences have been published, enabling a skilled worker to ligate them functionally with plasmid vectors (Siebwenlist et al., 1980). Certain genes from prokaryotes may be expressed efficiently in *E. coli* from their own promoter sequences, precluding the need for addition of another promoter by artifi-
20 cial means.

In addition to prokaryotes, eukaryotic microbes, such as yeast cultures may also be used, and here the promoter should be capable of driving expression. *Saccharomyces cerevisiase*, or
25 common baker's yeast is the most commonly used among eukaryotic microorganisms, although a number of other strains are commonly available. For expression in *Saccharomyces*, the plasmid YRp7, for example, is commonly used (Stinchcomb et al., 1979; Kingsman et al., 1979; Tschemper et al., 1980).
30 This plasmid already contains the trpl gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan for example ATCC No. 44076 or PEP4-1 (Jones, 1977). The presence of the trpl lesion as a characteristic of the yeast host cell genome then provides an
35 effective environment for detecting transformation by growth in the absence of tryptophan.

52

Suitable promoting sequences in yeast vectors include the pro-
moters for 3-phosphoglycerate kinase (Hitzman et al., 1980) or
other glycolytic enzymes (Hess et al., 1968; Holland et al.,
1978), such as enolase, glyceraldehyde-3-phosphate dehydro-
5   genase, hexokinase, pyruvate decarboxylase, phosphofructo-
kinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mu-
tase, pyruvate kinase, triosephosphate isomerase, phosphoglu-
cose isomerase, and glucokinase. In constructing suitable ex-
pression plasmids, the termination sequences associated with
10 these genes are also ligated into the expression vector 3' of
the sequence desired to be expressed to provide polyadenyla-
tion of the mRNA and termination.

Other promoters, which have the additional advantage of tran-
15 scription controlled by growth conditions are the promoter re-
gion for alcohol dehydrogenase 2, isocytochrome C, acid phos-
phatase, degradative enzymes associated with nitrogen metabo-
lism, and the aforementioned glyceraldehyde-3-phosphate dehy-
drogenase, and enzymes responsible for maltose and galactose
20 utilization. Any plasmid vector containing a yeast-compatible
promoter, origin of replication and termination sequences is
suitable.

In addition to microorganisms, cultures of cells derived from
25 multicellular organisms may also be used as hosts. In prin-
ciple, any such cell culture is workable, whether from verte-
brate or invertebrate culture. However, interest has been
greatest in vertebrate cells, and propagation of vertebrate in
culture (tissue culture) has become a routine procedure in re-
30 cent years (Tissue Culture, 1973). Examples of such useful
host cell lines are VERO and HeLa cells, Chinese hamster ovary
(CHO) cell lines, and W138, BHK, COS-7 293, *Spodoptera
frugiperda* (SF) cells (commercially available as complete ex-
pression systems from *i.a.* Protein Sciences, 1000 Research
35 Parkway, Meriden, CT 06450, U.S.A. and from Invitrogen), and
MDCK cell lines. In the present invention, an especially pre-
ferred cell line is S_2 available from Invitrogen, PO Box 2312,
9704 CH Groningen, The Netherlands.

Expression vectors for such cells ordinarily include (if ne-
cessary) an origin of replication, a promoter located in front
of the gene to be expressed, along with any necessary ribosome
5 binding sites, RNA splice sites, polyadenylation site, and
transcriptional terminator sequences.

For use in mammalian cells, the control functions on the ex-
pression vectors are often provided by viral material. For ex-
10 ample, commonly used promoters are derived from polyoma, Ade-
novirus 2, and most frequently Simian Virus 40 (SV40). The
early and late promoters of SV40 virus are particularly useful
because both are obtained easily from the virus as a fragment
which also contains the SV40 viral origin of replication (Fi-
15 ers et al., 1978). Smaller or larger SV40 fragments may also
be used, provided there is included the approximately 250 bp
sequence extending from the *Hind*III site toward the BglI site
located in the viral origin of replication. Further, it is
also possible, and often desirable, to utilize promoter or
20 control sequences normally associated with the desired gene
sequence, provided such control sequences are compatible with
the host cell systems.

An origin of replication may be provided either by construc-
25 tion of the vector to include an exogenous origin, such as may
be derived from SV40 or other viral (e.g., Polyoma, Adeno,
VSV, BPV) or may be provided by the host cell chromosomal re-
plication mechanism. If the vector is integrated into the host
cell chromosome, the latter is often sufficient.
30
Identification of useful IL5 analogues

It will be clear to the skilled person that not all variants
or modifications of native IL5 will have the ability to elicit
35 antibodies in an animal which are cross-reactive with the na-
tive form. It is, however, not difficult to set up an effec-
tive standard screen for modified IL5 molecules which fulfill
the minimum requirements for immunological reactivity dis-

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cussed herein. Hence, another part of the invention concerns a
method for the identification of a modified IL5 polypeptide
which is capable of inducing antibodies against unmodified IL5
in an animal species where the unmodified IL5 polypeptide is a
5 self-protein, the method comprising

-       preparing, by means of peptide synthesis or by molecular
        biological means, a set of mutually distinct modified IL5
        polypeptides wherein amino acids have been added to, in-
10   serted in, deleted from, or substituted into the amino
        acid sequence of an IL5 polypeptide of the animal species
        thereby giving rise to amino acid sequences in the set
        which comprise T-cell epitopes which are and foreign to
        the animal species, or preparing a set of nucleic acid
15   fragments encoding the set of mutually distinct modified
        IL5 polypeptides,
-       testing members of the set for their ability to induce
        production of antibodies by the animal species against
        the unmodified IL5, and
20 -   identifying and optionally isolating the member(s) of the
        set which significantly induces antibody production
        against unmodified IL5 in the animal species, or identi-
        fying and optionally isolating the polypeptide expression
        products encoded by members of the set of nucleic acid
25   fragments which significantly induces antibody production
        against unmodified IL5 polypeptide in the animal species.

In this context, the "set of mutually distinct modified IL5
polypeptides" is a collection of non-identical modified IL5
30 polypeptides which have e.g. been selected on the basis of the
criteria discussed above (e.g. in combination with studies of
circular dichroism, NMR spectra, and/or X-ray diffraction pat-
terns). The set may consist of only a few members but it is
contemplated that the set may contain several hundred members.
35 Likewise, the set of nucleic acid fragments is a collection of
non-identical nucleic acid fragments, each encoding a modified
IL5 polypeptide selected in the same manner.

55

The test of members of the set can ultimately be performed *in vivo*, but a number of *in vitro* tests can be applied which narrow down the number of modified molecules which will serve the purpose of the invention.

5

Since the goal of introducing the foreign T-cell epitopes is to support the B-cell response by T-cell help, a prerequisite is that T-cell proliferation is induced by the modified IL5. T-cell proliferation can be tested by standardized prolifera-

10 tion assays *in vitro*. In short, a sample enriched for T-cells is obtained from a subject and subsequently kept in culture. The cultured T-cells are contacted with APCs of the subject which have previously taken up the modified molecule and processed it to present its T-cell epitopes. The proliferation of

15 T-cells is monitored and compared to a suitable control (e.g. T-cells in culture contacted with APCs which have processed intact, native IL5). Alternatively, proliferation can be measured by determining the concentration of relevant cytokines released by the T-cells in response to their recognition of

20 foreign T-cells.

Having rendered highly probable that at least one modified IL5 of the set is capable of inducing antibody production against IL5, it is possible to prepare an immunogenic composition com-

25 prising at least one modified IL5 polypeptide which is capable of inducing antibodies against unmodified IL5 in an animal species where the unmodified IL5 polypeptide is a self-protein, the method comprising admixing the member(s) of the set which significantly induces production of antibodies in the

30 animal species which are reactive with IL5 with a pharmaceutically and immunologically acceptable carrier and/or vehicle and/or diluent and/or excipient, optionally in combination with at least one pharmaceutically and immunologically acceptable adjuvant.

35

Likewise, it is also possible to prepare an immunogenic composition which as an immunogen contains a nucleic acid fragment

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encoding a immunogenic IL5 analogue, cf. the discussion of nu-
cleic acid vaccination above.

The above aspects of the invention are conveniently carried
5 out by initially preparing a number of mutually distinct nu-
cleic acid sequences or vectors of the invention, inserting
these into appropriate expression vectors, transforming suit-
able host cells with the vectors, and expressing the nucleic
acid sequences of the invention. These steps can be followed
10 by isolation of the expression products. It is preferred that
the nucleic acid sequences and/or vectors are prepared by
methods comprising exercise of a molecular amplification tech-
nique such as PCR or by means of nucleic acid synthesis.

15

PREAMBLE TO EXAMPLES

Vaccine design

20 The exemplary candidates for an IL5 autovaccine are con-
structed according to the AutoVac™ concept (described in de-
tail in WO 95/05849) by substitution with known promiscuous T
cell epitopes into the human IL5 wild type protein. The sub-
stitutions are peptide substitutions, where the inserted pep-
25 tide may be of the same or different length than the deleted
peptide in the wild-type sequence.

For initial proof of concept by *in vivo* testing and screening,
it was decided to prepare the constructs in the murine IL5 se-
30 quence. By way of example, the tetanus toxoid epitopes P2 (SEQ
ID NO: 23) and P30 (SEQ ID NO: 24) are used as substituting
peptides, but any other suitable peptide containing or consti-
tuting a promiscuous T_H epitope could, according to the present
invention, be used.

35

It should be emphasized that the size of the molecule (115
res.) compared to the size of the substitutions (15 or 21
residues for P2 and P30, respectively) strongly limits the

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possible sites of structural non-destructive inserts. As the
disulfide bridges are important, but not imperative, for the
dimerization, some variants are made in pairs +/- elimination
of the cysteines.

5

In the construction of the candidate molecules, two basic pa-
rameters have been considered. First, it is attempted to con-
serve a maximum fraction of the three-dimensional structure of
the wild type hIL5, thereby conserving the native B-cell epi-
10 tope repertoire. This is supported by Dickason et al., (1994)
who demonstrated that IL5 B-cell epitopes known to be neutra-
lising are conformational. Conservation of the tertiary struc-
ture is sought achieved by introducing the modifications at
structurally "neutral" sites, such as loops or separate seg-
15 ments. The fact that the N-terminal helix "A" together with
the helices "B" and "C" are able to fold into a quaternary
structure with a second molecule, indicates that these 3 heli-
ces constitute a stable folding-scaffold.

20 Second, the biological activity in relation to the vaccine
concept has been considered. In general, an inactive construct
is preferable with a view to reducing putative toxic effects
of the molecules and in general for evaluating the immune re-
sponse. On the other hand, the optimum neutralising antibodies
25 should theoretically exhibit specificity for the part of IL5
that interacts with the IL5R. This is most likely achieved by
immunising with an active variant. Finally, it is not impossi-
ble that the biological effect of IL5 on the immune system
might act as an enhancer on the immune response, thus impro-
30 ving the overall effect. Based on Applicant's previous experi-
ences with other molecules, however, the majority of "theo-
retically possible active" constructs is expected to have low
or no activity.

35 Therefore, all variants suggested are potentially active but
can, if desirable, with relative ease be rendered inactive by
hindering the formation of the active dimer or by alterations

in the areas of the "A"- and "D"-helices that are involved in
the receptor binding/activation.

In summary, the above considerations of structure conservation
5 and biological activity defines the target areas as any one of
loops 1-3 as well as the C-terminal flexible area.

Loop 3 is selected as the primary target area since it is
structurally separated from the assumed tri-helical folding
10 scaffold. As it is furthermore possible to produce a biologi-
cally active monomer, by elongation of loop 3 (Dickason,
1996), this area holds the possibilities for generating all
types of variants: monomer/dimer and active/inactivated.

15 "Loop 1" is a second area containing a non-helical stretch of
a suitable length for substitutions. Variants from this region
would theoretically be active only if capable of dimerising,
but since the length of the wild-type loop makes it rather
flexible it is reasonable to expect a correct folding of the
20 protein after substitution.

Variants containing substitutions in the "loop 2" area will
also only be active as dimers. The area that can be substi-
tuted is short compared to the inserts and has a central posi-
25 tion in the assumed folding scaffold, two characteristics of
loop 2 which might be of hindrance to the correct folding of
the protein after substitution. On the other hand, loop 2 is
situated opposite to the area interacting with the IL5R, re-
sulting in an expected optimum presentation of the wild-type
30 neutralising epitopes if the modified protein is correctly
folded.

Finally, inserts in the C-terminal flexible region following
"helix D" are proposed. From a protein structure point of view
35 this concept appears fairly safe, but it is likely that modi-
fications in this region will affect both dimerization and
biological activity (if the modified protein is dimerized)

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since the C-terminal is located in the area of both receptor
binding and in the dimer interface.

The amino acid sequence of 10 variants initially constructed
5   according to the above considerations are set forth as SEQ ID
NOs: 2-11 and 13-22. Further variants constructed at a later
stage are set forth in SEQ ID NOs: 27-59 (including both DNA
nucleic acid sequences and amino acid sequences).

10 It should be noted, that all inserts except from the ones ac-
cording to Example 2 are prepared so as to include flanking
amino acid residues that are conserved from hIL5 to mIL5 in
order to promote the process of successful transfer of posi-
tive constructs from mice to man.

15

In the following examples, positions for substitution are in-
dexed according to the murine amino acid residue sequence num-
bers; the corresponding human positions are given in parenthe-
ses.

20

EXAMPLE 1

Variants with P2 substituting positions in loop 3 while pre-
25 *serving Cys84(86)*

The P2 epitope (SEQ ID NO: 23) is substituted into loop 3
while avoiding elimination of Cys84(86). These variants (SEQ
ID NOs: 2 and 28 (human), where amino acids 87-90 or 88-91 are
30 substituted and 13 and 46 (murine) where amino acids 85-88 pr
86-89 are substituted) are potentially active as both monomers
(due to the elongation of loop 3) and as dimers. SEQ ID Nos:
28 and 46 are also denoted hIL5.1 and mIL5.1, respectively.

35

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EXAMPLE 2

*Variants with P2 substituting positions in loop 1 while pre-
serving Cys42(44)*

5

The P2 epitope (SEQ ID NO: 23) is substituted into loop 1
while avoiding elimination of Cys42(44). These variants (SEQ
ID NOs: 3 and 36 (human) where amino acids 32-43 or 33-43 are
substituted and 14 and 56 (murine) where amino acids 30-41 or
10 31-41 are substituted) are potentially active as dimers only.
SEQ ID Nos: 36 and 56 are also denoted hIL5.5 and mIL5.5, re-
spectively.

15 EXAMPLE 3

Variants with P2 substituting positions in loop 2

The P2 epitope (SEQ ID NO: 23) is substituted into loop 2.
20 These variants (SEQ ID NOs: 4 and 34 (human) where amino acids
59-64 are substituted and 15 and 50 (murine) where amino acids
57-62 are subsituted) are potentially active as dimers only.
SEQ ID Nos: 34 and 50 are also denoted hIL5.4 and mIL5.4, re-
spectively.

25

EXAMPLE 4

Variants with P2 substituting positions in loop 3 while elimi-
30 *nating Cys84(86)*

The P2 epitope (SEQ ID NO: 23) is substituted into loop 3
while *eliminating* Cys84(86). These variants (SEQ ID NOs: 5 and
38 (human) where amino acids 86-91 are substituted and 16 and
35 54 (murine) where amino acids 84-89 are substituted) are in
principle similar to the variants of type #1 (SEQ ID NOs: 2
and 28 and 13 and 46), but the generation of monomer products
has been facilitated by inhibiting the formation of disulfide

bridging and adjusting the length of loop 3. SEQ ID Nos: 38
and 54 are also denoted hIL5.6 and mIL5.6, respectively.

5 EXAMPLE 5

*Variants with P2 substituting positions 108-111(110-113) in
the C-terminus*

10 The P2 epitope (SEQ ID NO: 23) is substituted into the
C-terminal area succeeding helix D. These variants (SEQ ID
NOs: 6 and 17) are potentially active as a dimer only.

15 EXAMPLE 6

*Variants with P30 substituting positions in loop 3 while pre-
serving Cys84(86)*

20 The P30 epitope (SEQ ID NO: 24) is substituted into loop 3
avoiding elimination of Cys84(86). These variants (SEQ ID NOs:
7 and 40 (human) where amino acids 88-91 or 87-90 are substi-
tuted and 18 and 58 (murine) where amino acids 85-88 or 86-89
are substituted) are potentially active both as monomers (due
25 to the elongation of loop 3) and as dimers. SEQ ID Nos: 40 and
58 are also denoted hIL5.7 and mIL5.7, respectively.

EXAMPLE 7

30

*Variants with P30 substituting positions in loop 1 while pre-
serving Cys42(44)*

The P30 epitope (SEQ ID NO: 24) is substituted into loop 1,
35 avoiding elimination of Cys42(44). These variants (SEQ ID NOs:
8 and 30 (human) where amino acids 32-43 are substituted and
19 and 48 (murine) where amino acids 30-41 are substituted)

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are potentially active as dimers only. SEQ ID Nos: 30 and 48
are also denoted hIL5.2 and mIL5.2, respectively.

5 EXAMPLE 8

Variants with P30 substituting positions in loop 2

The P30 epitope (SEQ ID NO: 24) is substituted into loop 2.
10 These variants (SEQ ID NOs: 9 and 20 where amino acids 59-64
and 57-62 are substituted, respectively) are potentially ac-
tive as dimers only.

15 EXAMPLE 9

Variants with P30 substituting positions in the C terminus

The P30 epitope (SEQ ID NO: 24) is substituted into the
20 C-terminal area succeeding helix D. These variants (SEQ ID
NOs: 10 and 21 where amino acids 110-113 and 108-111 are sub-
stituted, respectively) are potentially active as dimers only.

25 EXAMPLE 10

*Variants with P2 substituting positions 84-89 (86-91) and P30
substituting positions 110-113*

30 The P2 epitope (SEQ ID NO: 23) is substituted into loop 3
eliminating Cys84(86) and the P30 epitope (SEQ ID NO: 24) is
substituted into the C-terminal area succeeding helix-D. These
variants (SEQ ID NOs: 11 and 22) are together with variants of
type #12 the only ones containing both epitopes and are poten-
35 tially active monomers.

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EXAMPLE 11

*Variants with P30 substituting positions in loop 3 while
eliminating Cys84(86)*

5

The P2 epitope (SEQ ID NO: 24) is substituted into loop 3
while *eliminating* Cys84(86). These variants (SEQ ID NOs: 42
(human) where amino acids 86-91 are substituted and 58 (mu-
rine) where amino acids 84-89 are substituted) are in princi-
10 ple similar to the variants of type #6, but the generation of
monomer products has been facilitated by inhibiting the forma-
tion of disulfide bridging and adjusting the length of loop 3.
SEQ ID Nos: 42 and 58 are also denoted hIL5.12 and mIL5.12,
respectively.

15

EXAMPLE 12

Variants with P2 and P30 substituting positions in loop 3

20

The P2 (SEQ ID NO: 23) and P30 (SEQ ID NO: 24) epitopes are
substituted into loop 3 while preserving Cys84(86). These
variants (SEQ ID NOs: 44 and 60 where amino acids 88-91 and
86-89 are substituted, respectively) contain both epitopes and
25 are potentially active monomers. SEQ ID NOs: 44 and 60 are
also denoted hIL5.13 and mIL5.13, respectively.

EXAMPLE 13

30

Choise of expression system

Recombinant IL5 has been expressed in a number of different
expression systems including yeast, insect cells and CHO cells
35 (Tavernier *et al.*, 1989).

According to the present invention, one suitable expression
system is *E. coli*, based on previous studies reporting the use

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of this host for production hIL5 (Proudfoot *et al.*, 1990, Graber *et al.*, 1993). The recombinant protein is expressed as inclusion bodies that are converted into the biologically active dimer upon purification and re-folding (e.g. using the
5 generally applicable refolding methods disclosed in US 5,739,281). The speed and simplicity of *E. coli* expression allows immediate initiation of the production of protein when the genetic constructs are ready, thus facilitating rapid generation of material to establish an *in vivo* proof of the IL5
10 autovaccine concept.

If for some reason the feasibility is found to be to low (e.g. low yield following re-folding, instability of the products or improved pharmacokinetical parameters related to glycosylation
15 etc), production in yeast could be considered in a further development of the autovaccine.

Recently, promising results have been obtained using the *Drosophila melanogaster* expression system using S_2 cells (avail-
20 able from Invitrogen) and at present this system is the preferred embodiment for expression of the IL5 analogues of the invention.

IL5 variant protein was produced from S2 drosophila cells
25 stably expressing the IL5 constructs. Several different transfection methods were tested, and both Ca_2PO_4 and Lipofectin were chosen. Two different subclones of S2 cells were used and transfected with Ca_2PO_4 and Lipofectin, respectively. The two clones were obtained from ATCC and Lars Søndergaard of the
30 University of Copenhagen, respectively. Using both methods suitable stable lines were selected expressing mIL5 and mIL5.1 proteins in the 2-10 mg/L range.

Materials & Methods:

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S2 cells were grown and maintained in Schneider's medium (Sigma) containing 5-10% fetal calf serum (FCS), 0.1% pluronic

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F68 (Sigma), penicillin/streptomycin (Life Technologies) grown
in shake flasks at 25°C and 120 rpm.

Lipofectin transfections were performed in 250 ml or 1 l shake
5   flasks. S2 cells were split to 2.5-3 x 10^6/ml into 50 ml Ex-
cell 420 (JRH Biosciences) without antibiotics, and grown
overnight in a 250 ml shake flask. The next morning the Li-
pofectin reagents were prepared: tube 1) 300-1200 µg plasmid
DNA containing the gene of interest, plus 15-60 µg pCoHYGRO
10 hygromycin selection plasmid (20:1 ratio of plasmids) in 15-45
ml serum and supplement-free medium; tube 2) 1ml Lipofectin in
5 ml serum and supplement-free medium. After 1 hour at room
temperature, tubes 1 and 2 were mixed and rested for 15 mi-
nutes at room temperature before gently adding to S2 cells.
15 After growing cells overnight new media was added containing
full suplements plus 150-300 µg/ml Hygromycin.

Transient and stable lines were induced with either 500 µM
copper sulfate or 10 µM cadmium chloride for 48-72 hours in
20 serum-free Ex-cell 420 medium (JRH Biosciences).

Results:

33 stable lines were generated by Ca$_2$PO$_4$ and 23 by Lipofectin.
25 The expression yields varied from non-detectable up to 11
mg/L. The following table summarizes a few of the lines used
for protein production.

Expression result summary from best mIL5 S2 cell transfections.

30

Plasmid	Construct	S2 cells	Transfection Method	Yield
p612	IL5/His15/mIL5wt	ATCC	Ca$_2$PO$_4$	3.5 mg/L
p767	Bip/His15/mIL5wt	LS	Lipofectin	11 mg/L
p613	IL5/His15/mIL5.1	ATCC	Ca$_2$PO$_4$	2.6 mg/L
p768	Bip/His15/mIL5.1	ATCC	Ca$_2$PO$_4$	0*
p614	IL5/His15/mIL5.5	LS	Lipofectin	0*

* Expression plasmid contained sequence mutations.

Hence, S2 cells can be transfected by either calcium phosphate precipitation or Lipofectin. Due to the difference in expression level between plasmids p612 and p767, it seems that the Bip signal peptide is a more efficient leader sequence than
5 the endogenous mIL5 leader in S2 cells.

EXAMPLE 14

10 *Screening and selection of the modified molecules*

Following expression, the recombinant protein is purified and characterised. The characterisation of the autovaccine candidates will include analytical chromatography, iso-electric fo-
15 cussing (IEF), SDS-PAGE, amino acid composition analysis, N-terminal sequence analysis, mass spectrometry, low angle laser light scattering, standard spectroscopy, and Circular Dichroism to an extent that precisely document the relevant parameters defining the intended protein product.
20
The His tagged proteins have been purified using a two-step procedure until recently. However, the yield and purity were not as high as expected after the final chelate-step. A new one-step purification procedure has been implied with 3 major
25 advantages achieved: higher yield, higher through-put and higher purity of the final product. Cleavage conditions for removal of the histag have also been established.

The two-step IL5 purification procedure:
30
Expression of the protein is induced by addition of metal ions to the media. These metal-ions have to be removed before application of the protein to the chelate column. Thus, a total of 20 mM EDTA is added to complex the metal-ions and the su-
35 pernatant is then passed over a SP-sepharose column to capture the protein. After washing to remove unbound protein, bound

67

protein is eluted by a step-gradient of NaCl. This step serves
two purposes: a concentrating step reducing the volume by a
factor of 30, and buffer-exchange.

5 Relevant fractions (as determined by SDS-PAGE) are pooled and
further purified on the metal chelate column.

The protein is applied to a Ni^{2+}-charged chelate column and un-
bound protein washed off. Bound protein is then eluted using
10 an Imidazole gradient. All fractions, flow-through and EDTA-
washes of the column, are then checked by both SDS-PAGE and
dot-blot.

Relevant fractions (as determined by SDS-PAGE and dot-blot)
15 are pooled and dialyzed twice against 10 X volume of PBS, pH
adjusted to 6.9.

After filtration, the dialyzed material is concentrated until
a suitable concentration is achieved (preferably 1 mg/ml). Fi-
20 nally, the protein is aliqouted and stored at -20°C.

The following specific protocol has been applied:

1)   The received supernatant is centrifuged at 2500 x g for 15 min
25   (if infection has occurred, it needs centrifugation at 22000 x g
        for 30 min. The supernatant is then filtered using a 0.45 μm
        filter followed by a 0.22 μm filter (sometimes it is necessary
        to filter through a 5 μm filter first).
        The supernatant is then mixed 1:1 with buffer A (see step 2)
30   containing 40 mM EDTA, resulting in a final buffer composition
        of 0.2 M NaH_2PO_4, 10% glycerol, 20 mM EDTA, pH 6.0

2)   The filtered supernatant is subsequently applied to a SP-Sepha-
        rose column equillibrated in buffer A. A total of 1-2 L (depen-
35   ding on protein concentration, the above holds for 1-10 mg IL5
        /L) can be applied to an 80 ml column. Flow during application:

68

1-2 ml/min (usually over night), the flow-through is collected
and saved for later analysis. Following application, the column
is washed with 2-3 column volumes (CV) of A-buffer until a sta-
ble baseline is achieved. Bound protein is eluted using a step
5   gradient: 0-100-500-1000 mM NaCl, fractions of 10 ml are col-
lected, flow is 10 ml/min. Purification is performed at 5°C.

The column is cleaned with 2 CV 1 M NaOH, flow 5 ml/min after
each run and re-equilibrated in buffer A.
10

Buffer A: 0.2 M NaH_2PO_4, 10% glycerol, pH 6.0
Buffer B: 0.2 M NaH_2PO_4, 1 M NaCl, 40 mM Imidazole, 10% gly-
            cerol, pH 6.0

15   The same procedure is used for both wt and variants.

All fractions, starting material and flow-through are tested in
dot-blot and SDS-PAGE. The fractions containing IL5 are pooled
and further purified using a chelate-column.
20

The one-step IL5 purification procedure:

The supernatant is applied directly to a 70-ml chelate-column
charged with $ZnCl_2$. After removal of the unbound material by
25 washing, bound protein (IL5 and contaminants) is eluted by ap-
plying a gradient of Imidazole. This method takes full advan-
tage of the His tag giving a one-step purification procedure
with a high degree of purity of the final product (>95%).
Relevant fractions (as determined by SDS-PAGE and dot-blot)
30 are pooled and dialyzed twice against 10 X volume of PBS, pH
adjusted to 6.9 and concentration of NaCl adjusted to 400 mM.

After filtration, the dialyzed material is concentrated until
a suitable concentration is achieved (preferably 1 mg/ml). Fi-
35 nally, the protein is aliqouted and stored at -20°C.

A specific protocol follows the following steps

1) The supernatant is filtered through a 0.45 µm filter to remove
    impurities and diluted 1:1 with buffer A.

5   A 70-ml Fast Flow chelate column is rinsed with 5 CV water and
        then charged with 10 CV 10 mM $ZnCl_2$, pH 7. After equilibration
        with 5 CV A-buffer, the sample is applied using the pump (flow
        10 ml/min). The flow-through is collected and saved for later
        analysis. Bound protein is eluted using an Imidazole-gradient
10   going from 0 to 250 mM Imidazole over 30 CV. Finally, the column
        is stripped by 5 CV of buffer C.
        Fractions of 10 ml are collected.

        Buffers:
15   A: 20 mM NaH_2PO_4, 0.5 M NaCl, 10% glycerol, pH 7.
        B: 20 mM NaH_2PO_4, 0.5 M NaCl, 10% glycerol, pH 7, 0.25 M imida-
           zole
        C: 20 mM NaH_2PO_4, 0.5 M NaCl, 0.1 M EDTA pH 7.0.

20   All fractions, flow-through and starting material is tested in
        SDS-PAGE.

2) The purest fractions (as determined by SDS-PAGE) containing IL5
    are pooled (50 µl are saved for later analysis) and dialyzed
25   twice against 10 X volume of PBS, pH adjusted to 6.9. at 6°C,
        MWCO 12-14 kDa. The dialysate is filtered through a 0.22 µm fil-
        ter (50 µl is saved for later analysis) and A_{280} is measured u-
        sing dialysis-buffer (filtered through 0.45 µm) as reference.
        The volume before and after dialysis is measured and samples
30   showing the dialysis/concentrating step are saved for later
        analysis by SDS-PAGE (after step 3).

3) NaCl is added to the dialyzed protein until a total concentra-
    tion of 400 mM and it is then concentrated using either an Ami-
35   con apparatus (for volumes larger than 50 ml) or Vivaspin con-
        centrating device (for 10-50 ml). In both cases, the membrane is
        saturated with 10 ml PBS-buffer buffer before the sample is ap-
        plied. The sample should be concentrated until a concentration
        of preferably 1 mg/ml is achieved (as measured by A_{280}). The dia-
40   lyzed, concentrated sample is filtered through a 0.22 µm filter

and marked with an E-nr. The A_{280} is measured using the flow-
through as reference.

All samples from the dialysis and concentrating step are ana-
5   lyzed by SDS-PAGE and Coomassie-stained. The purified protein is
stored frozen in aliquots and a sheet describing the sample is
filed in the "IL5-protein"-folder.

The above-described procedure gives a protein with a purity of
10 approximately 90-95%, still containing the His Tag.
When sequenced, both IL5wt and variant IL5.1 gave the expected
N-terminal sequences including the His Tag.

The   purification procedure referred to above has been imple-
15 mented in the following specific setup:

1) The pooled fractions from the SP-sepharose column are filtered
through a 0.45 μm filter to remove impurities.

20   A 5-ml HiTrap chelate column(use only dedicated columns) is
rinsed with 15 ml water (using a syringe) and then charged with
15 ml 0.1 M NiSO₄ and washed with 15 ml water. The column is con-
nected to the Äkta-system and equilibrated with 2-3 CV A-buffer.
The sample is applied using either the loop or pump - depending
25   on the volume (flow 4 ml/min), the flow-through is collected and
saved for later analysis. Bound protein is eluted using an Imi-
dazole-gradient going from 0 to 500 mM Imidazole over 20 CV.
Fractions of 5 ml are collected. Finally, the column is stripped
using 5 CV of buffer B2.
30
Buffer A: 0.2 M NaH₂PO₄, 0.5 M NaCl, 10 % glycerol, pH 5.0
Buffer B1: 0.2 M NaH₂PO₄, 0.5 M NaCl, 0.5 M Imidazole, 10 % gly-
cerol, pH 5.0
Buffer B2: 50 mM Na-acetate, 0.5 M NaCl, 0.1 M EDTA, 10 % gly-
35   cerol, pH 4.5

All fractions, flow-through and starting material are tested in
dot-blot, all relevant fractions are tested in SDS-PAGE.

2) The purest fractions (as determined by SDS-PAGE) containing IL5
   are pooled (save 50 µl for later analysis) and dialyzed twice
   against 10 X volume of PBS, pH adjusted to 6.9. at 6 °C, MWCO 12-
   14 kDa. The dialysate is filtered through a 0.22 µm filter (save

5   50 µl for later analysis) and A_{280} is measured using filtered di-
   alysis-buffer as reference. The volume before and after dialysis
   is measured and samples showing the dialysis/concentrating step
   are saved for later analysis by SDS-PAGE (after step 3).

10 3) After addition of extra NaCl up to a final concentration of 400
       mM, the dialyzed protein is concentrated using either an Amicon
       apparatus (for volumes larger than 50 ml) or Vivaspin concen-
       trating device (for 10-50 ml). In both cases, the membrane is
       saturated with 10 ml PBS buffer before the sample is applied.

15   The sample should be concentrated until a concentration of pre-
       ferably 1 mg/ml is achieved (as measured by A_{280}). The A_{280} is
       measured using the flow-through as reference. The dialyzed, con-
       centrated sample is filtered through a 0.22 µm filter and marked
       with an E-nr.

20

All samples from the dialysis and concentrating step are ana-
lyzed by SDS-PAGE and Coomassie-stained. The purified protein
is stored frozen in aliquots.

25

Other purification procedures that have been evaluated are:

Zn^{2+}-chelate purification: Elution of the protein using an in-
creasing Imidazole gradient has proved very efficient as the

30 wt-protein binds strongly to the column. The Drosophila super-
natant can be directly applied and after washing, the IL5wt
can be eluted by Imidazole. The column is charged with 10 CV
10 mM $ZnCl_2$, and washed with water. The pH of the binding and
elution buffers has to be above 6.5 as otherwise the $ZnCl_2$ will

35 precipitate.

72

Con A affinity chromatography is under investigation. The possibility of using the glycosylation present on IL5 as an affinity-tag and elute by application of a monosaccharide-analog would be interesting since it could be applied to the non-His
5 tagged constructs as well.

Removal of Histag:

Removal of the 15 aa His tag (SEQ ID NO: 25) has been per-
10 formed according to suppliers (Unizyme) instructions:

        The purified and dialyzed/concentrated His tagged IL5 is de-His
        tagged by the sequential addition of two enzymes, DAP1 and Glutamine
        cyclotransferase. DAP1 removes two amino acids from the free N-ter-
15   minus while the QCT

        The enzyme needs to be activated first:
        9 µl HT-DAP1 (10 U/ml) is mixed with 9 µl 20 mM cysteamine-HCl. Af-
        ter 5 min incubation at room temperature, a total 108 µl HP-GCT (100
20   U/ml) and 54 µl TAGZyme buffer is added. This must be used within 15
        min.

        This portion will digest 1 mg of His tagged protein.

25   The His tagged protein is mixed with 150 µl activated enzyme and in-
        cubated at 37°C for 120 min. Samples are withdrawn for SDS-PAGE
        analysis (10 µl) after 0, 10, 30, 60 and 120 min. The samples are
        put on ice to stop the digestion.

30   **Buffers**:

        1. TAGZyme buffer: 20 mM NaPO$_4$ buffer, pH 7.5; 150 mM NaCl
        2. 20 mM Cysteamine-HCl

35   The digested protein (as determined from SDS-PAGE analysis or N-ter-
        minal sequencing) is applied to a 1-ml Ni-chelate column equili-
        brated in PBS. Everything is collected.

The flow-through from the application is saved for later analysis.
The column is eluted by addition of 3 CV PBS, fractions of 0.5 ml
are collected. The column is cleaned by washing with 2 CV 0.5 M Imi-
dazole, and fractions are saved for analysis.

5

All fractions are tested in SDS-PAGE, and fractions containing IL5
are pooled and A_{280} is measured using PBS as reference. Finally, the
protein is concentrated using a Vivaspin concentrating device until
a concentration of 1 mg/ml is achieved.

10

Removal of His tag has been performed in small-scale experi-
ments (0.1-1 mg) and has not been up-scaled. It should be
noted that removal of the tag requires an unblocked and non-
modified N-terminus.

15

The His tagged protein is incubated with two enzymes, a dipep-
tidyl amino peptidase which removes two amino acids at a time
and a glutamic acid cyclotransferase which catalyze the con-
version of a glutamic acid into a pyro-glutamic acid. This
20 conversion blocks further degradation by the dipeptidyl amino
peptidase. The digestion mixture is then passed through a che-
late column which should retain the enzymes (which are His
tagged), contaminating proteins binding to the column and non-
degraded or partially degraded protein. The de-tagged protein
25 passes the column and is collected in the flow-through. After
a second digestion with an enzyme that removes the pyro-glu-
tamic acid, the protein is again passed over a chelate-column
to remove the second enzyme. It is expected that the protein
needs to be concentrated again at this final stage.

30

General observations:

The pI of UniHis-IL5wt is 9.5 and the optimum pH-value for the
protein seems to be 6.5-7.0 (has not been investigated tho-

74

roughly). A NaCl-concentration of 400 mM seems to stabilize
the protein during concentration.

EXAMPLE 15

5

In vitro screening

The primary *in vitro* screening will be in the form of an en-
zyme-linked immunosorbent assay (ELISA): A competitive ELISA
10 towards wild-type IL5 provides an estimate of the presence of
relevant B-cell epitopes in the modified IL5 constructs before
introduction thereof into animals.

A conventional ELISA assay can be used to measure titres of
15 auto-antibodies in the serum of vaccinated animals. Antibodies
(both mono-specific and monoclonal) towards the human as well
as towards the murine IL5 are commercially available from R&D
Systems, 614 McKinley Place NE, Minneapolis, MN 55413, USA.

20 The biological activity of the product and/or the neutralising
capacity of induced auto-antibodies can be tested in an IL5
bioassay. Previously reported examples of such bioassays are:
Assessment of IL5 induced proliferation of TF1 cells (for hu-
man IL5) and assessment of IL5 induced proliferation of BCL1
25 cells or B13 B cells (for murine IL5) (Callard & Gearing 1994,
Dickason *et al.*, 1994).

The effect on airway responsiveness of the autovaccine can
also be tested in an *in vitro* assay wherein the trachea from
30 vaccinated mice are removed and placed on a hook in an organ
bath. The tension of the trachea after histamine challenge is
measured (van Oosterhout *et al.*, 1995).

Consequently, in order to be able to determine the biological
35 activity of recombinant mIL5 (and mIL5 AutoVac) protein sam-
ples, a cellular bioactivity assay for murine IL5 is being es-
tablished. The assay is based on the ability of the B cell
lymphoma line BCL1 to proliferate in response to mIL5 added to

the culture medium. Two different BCL1 clones were obtained from ATCC, BCL1 clone 5B1b (ATCC CRL-1669) and BCL1 clone CW13.20.3B3 (ATCC TIB-197).

5 In a typical BCL1 proliferation experiment, the cells are plated in complete RPMI medium supplemented with fetal calf serum (FCS) in microtiter plates and incubated with dilution series of murine IL5. Proliferation of the BCL1 cells is measured by incorporation of tritiated thymidine. Several optimi-
10 zation experiments have been performed using dilution series of purchased recombinant mIL5 (R&D Systems) for stimulation. The variable parameters include: incubation intervals, ^{3}H-thymidine pulsing intervals, numbers of cells plated per well, fetal calf serum (FCS) concentrations and concentrations of
15 added mIL5. Dose-dependent proliferation of the BCL1 cells with a maximal proliferation of about 3 times the background (BCL1 cells with no mIL5 added) has been obtained.

The BCL1 assay has been used to determine the biological ac-
20 tivity of the following samples expressed from Drosophila S2 cells and purified as described above: HIS-mIL5wt material (E1320), HIS-mIL5wt material (E1422), HIS-mIL5.1 material (E1396) and an "S2-background-preparation" (E0016). The proliferation in response to one HIS-mIL5wt (E1320) preparation
25 was significantly higher than the proliferation in response to the "S2-background-preparation", whereas the mIL5.1 variant and one wild type preparation (E1422) were determined as biologically inactive.

30 Ongoing work includes inhibition of the BCL1 proliferation with anti-mIL5, and the anti-mIL5 monoclonal antibody TRFK5 is used for optimization studies. This is done in order to use this assay to determine the ability of anti-mIL5 antisera from immunised mice to inhibit the biological activity of mIL5.

EXAMPLE 16

5 *In vivo models*

For measuring the *in vivo* effect of the autovaccine,
well-known animal models for asthma exists. Normally, the ani-
mal is sensitised with a compound (allergen/antigen) and after
10 challenge with the aerosolised compound, broncho-constriction
(airway conduction) is measured using a body plethysmograph.
The eosinophil cell counts in the BAL fluid are also deter-
mined.

15 Several of the studies investigating the effect of anti-IL5
mAb's have been successfully performed in mice. Against use of
the murine model speaks the fact the IL5 acts as a B-cell
growth factor, rendering possible interference with the murine
antibody response. However, as shown in a study using IL5
20 knock-out mice, the T-cell dependent antibody response against
ovalbumin as well as cytotoxic T-cell development appeared
normal (Kopf *et al.*, 1996). As the mouse is also the most
practical and economical model in comparison to guinea pigs or
monkeys, the ovalbumin sensitised Bal/c mice model of
25 asthma/airway hypersensitivity as used by Hamelman et al.
(1997) will be used.

If, however, the effect of IL5 on B-cells in the murine model
turns out to be a problem, the use of other suitable animal
30 models known in the art will be applied.

77

EXAMPLE 17

Preparation of DNA constructs encoding murine IL5 and variants thereof

5
<u>Construction of variants in pcDNA3.1+:</u>
Insertion of P2 and P30 epitopes into wildtype mIL5 was done
by SOE-PCR with overlapping primers containing the epitope se-
quences. Wildtype mIL5 gene including leader sequence (SEQ ID
10 NO: 63), cloned into pcDNA3.1+ with consensus Kozak sequence
(obtaining plasmid p815), was used as template for the PCR re-
actions. The resulting fragments were digested with *Nhe*I and
*Not*I, purified and cloned into p815 was used as template for
the PCR.

15

<u>Cloning of variants into pMT Drosophila vector with BiP leader</u>
<u>and UNI-His tag:</u>
Wildtype mIL5 was cloned into the pMT *Drosophila* expression
vector series (Invitrogen) by generating a PCR fragment with
20 mIL5 specific primers containing appropriate restriction sites
and, in addition, containing sequences encoding a Drosophila
Kozak like sequence followed by the Drosophila BiP leader se-
quence followed by a sequence encoding a UNI-HIS tag (SEQ ID
NO: 25) fused to the 5' end of the sequence encoding mature
25 mIL5. Wildtype mIL5 cDNa sequence was used as template. The
resulting fragment was digested with *Eco*RI and *Not*I and was
subsequently cloned into the pMT/V5-HisA vector (Invitrogen).
The resulting plasmid (p818) was used for cloning of epitope
containing variants into pMT. These were cloned by digesting
30 the variants made in pcDNA3.1+ with *Sac*I and *Not*I and cloning
the resulting fragments into p818.

Cloning of variants into pAC5:

Wildtype and variants of mIL5 were cloned into the pAC5 con-
stitutive Drosophila expression vector by digestion of vari-
ants in pMT with *EcoRI* and *NotI* and cloning the resulting
5 fragments into the pAC5.1/V5-HisA vector (Invitrogen).

EXAMPLE 18

10 *Preparation of DNA constructs encoding human IL5 and variants*
thereof

Five lines of plasmids are contemplated containing unmodified
IL5 and all or some of the nine IL5 variants. The lines in-
15 clude: 1) human IL5 for DNA vaccination in the pCI vector
suited for expression in human cells, 2) human IL5 with the
BiP leader sequence and a 15 aa His tag (SEQ ID NO: 25, ob-
tained from UNIZYME in Hørsholm, Denmark. The tag is termed
"UNI" or "UNI-His tag" herein) in the pMT/V5/HIS vector for
20 inducible expression in Drosophila, 3) as in 2 but without the
His tag, 4) as in 3 but with murine IL5 and 5) human IL5 with
the DAPI leader sequence and the 15 aa HIS tag in the vector
pVL1393 for expression in the baculo-virus system.

Plasmids for DNA-vaccination in the pCI vector:

Name	ref #	Strain #	Epitope
hIL5 (pCI)	p888	MR#1237	none
hIL5.1 (pCI)	p889	MR#1238	P2, Loop 3
hIL5.2 (pCI)	p890	MR#1239	P30, Loop 1
hIL5.3 (pCI)	p891	MR#1240	P30, Loop 2
hIL5.4 (pCI)	p892	MR#1241	P2, Loop 2
hIL5.5 (pCI)	p893	MR#1242	P2, Loop 1
hIL5.6 (pCI)	p894	MR#1243	P2, Loop 3
hIL5.7 (pCI)	p895	MR#1244	P30, Loop 3
hIL5.12 (pCI)	p896	MR#1245	P30, Loop 3
hIL5.13 (pCI)	p897	MR#1246	P2 and P30, Loop 3

5 Plasmids for human IL5 expression in Drosophila with the UNI-
HIS tag and BiP leader sequence in pMT/V5/HIS :

Name	Ref #	Strain #	Epitope
hIL5m-UNI-BiP (pMT/V5-HisA)	p899	MR#1247	none
hIL5.1m-UNI-BiP (pMT/V5-HisA)	p900	MR#1248	P2, Loop 3
hIL5.2m-UNI-BiP (pMT/V5-HisA)	p901	MR#1249	P30, Loop 1
hIL5.3m-UNI-BiP (pMT/V5-HisA)	p929	MR#1277	P30, Loop 2
hIL5.4m-UNI-BiP (pMT/V5-HisA)	p902	MR#1250	P2, Loop 2
hIL5.5m-UNI-BiP (pMT/V5-HisA)	p903	MR#1251	P2, Loop 1
hIL5.6m-UNI-BiP (pMT/V5-HisA)	p904	MR#1252	P2, Loop 3
hIL5.7m-UNI-BiP (pMT/V5-HisA)	p905	MR#1253	P30, Loop 3
hIL5.12m-UNI-BiP (pMT/V5-HisA)	p906	MR#1254	P30, Loop 3
hIL5.13m-UNI-BiP (pMT/V5-HisA)	p907	MR#1255	P2 and P30, Loop 3

10 Plasmids for human IL5 expression in Drosophila with the BiP
leader sequence, but without the UNI-HIS tag in pMT/V5/HIS:

80

Name	Ref #	Strain #	Epitope
hIL5m-BiP (pMT/V5-HisA)	p908	MR#1256	none
hIL5.1m-BiP (pMT/V5-HisA)	p909	MR#1257	P2, Loop 3
hIL5.2m-BiP (pMT/V5-HisA)	p921	MR#1269	P30, Loop 1
hIL5.3m-BiP (pMT/V5-HisA)	p922	MR#1270	P30, Loop 2
hIL5.4m-BiP (pMT/V5-HisA)	p923	MR#1271	P2, Loop 2
hIL5.5m-BiP (pMT/V5-HisA)	p924	MR#1272	P2, Loop 1
hIL5.6m-BiP (pMT/V5-HisA)	p925	MR#1273	P2, Loop 3
hIL5.7m-BiP (pMT/V5-HisA)	p926	MR#1274	P30, Loop 3
hIL5.12m-BiP (pMT/V5-HisA)	p927	MR#1275	P30, Loop 3
hIL5.13m-BiP (pMT/V5-HisA)	p928	MR#1276	P2 and P30, Loop 3

Plasmids for murine IL5 expression in Drosophila with the BiP
leader sequence, but without the 15 aa His tag in pMT/V5/HIS:

5

Name	ref #	Strain #	Epitope
mIL5m-BiP (pMT/V5-HisA)	p918	MR#1266	none
mIL5.1m-BiP (pMT/V5-HisA)	p919	MR#1267	P2, Loop 3
mIL5.2m-BiP (pMT/V5-HisA)	p920	MR#1268	P30, Loop 1

Plasmids for human IL-5 expression in the baculo-virus system
with the UNI-HIS tag and DAP1 leader sequence pVL1393 in
10 pVL1393:

Name	Ref #	Strain #	Epitope
hIL5m-UNI-DAP1 (pVL1393)	p916	MR#1264	none
hIL5.1m-UNI-DAP1 (pVL1393)	p917	MR#1265	P2, Loop 3

EXAMPLE 19

DNA immunization studies

5 Generation of vectors encoding mIL5wt, mIL5.1 and mIL5.5 with
Kozak sequences for DNA vaccination experiments:
DNA fragments encoding mIL5wt, mIL5.1 and mIL5.5 including the
natural leader sequence (SEQ ID NO: 63) were inserted into
pcDNA3.1 thus yielding new plasmids p521, 522, and p523. In
10 order to enhance expression of cDNA in mammalian cells, Kozak
concensus sequences were inserted upstream of the coding se-
quences using PCR technology. PCR reactions were performed u-
sing p521, p522 and p523 as templates and a forward primer en-
coding the Kozak sequence immediately upstream of the mIL5
15 leader start codon. Purified PCR products were cloned into
pcDNA3.1+ vector using restriction endonucleases *Bam*HI and
*Not*I. The resulting plasmids p815, p816 and p817, respec-
tively, were verified by DNA sequencing. All other plasmids
used for DNA vaccination experiments were constructed using
20 the p521 plasmid as starting material.

In vitro translation of DNA vaccination plasmids using Promega
Kit:
A commercial kit using rabbit reticulocyte extract to generate
25 in vitro translated protein product plasmid DNA, has previ-
ously been successfully used in our lab to monitor protein ex-
pression from pcDNA plasmid encoding e.g ovalbumin cDNA. Mu-
rine IL5 DNA vaccination plasmids were added to the kit re-
agents according to the standard procedure. However, several
30 attempts to detect expressed mIL5 material on autoradiograms
failed whereas positive controls worked. Results from COS cell
transfections and DNA vaccination shows that the gene products
are expressed, so we did not investigate these technical pro-
blems further.

Transient transfection of COS cells with DNA vaccination plasmids to determine expression levels:

In order to monitor the transfection/expression efficiency of
5 the plasmids used for DNA vaccination experiments, a transient
transfection assay using COS cells was established. COS cells
were trypsinized and plated in DMEM medium supplemented with
10 % FCS in T25 culture flasks. The cells were transfected at
day 2 using the Dotap kit (Roche Diagnostics) and harvested at
10 day 5. Culture supernatant, whole cell lysate and membrane en-
riched preparations were tested in Western blotting to detect
anti-mIL5 reactive expression product. The anti-mIL5 reactive
product in the cell preparations consistently migrated as 2-3
separate bands of 21-28 kD in SDS-PAGE, whereas the MW of the
15 mIL5 monomer used as standard (expressed in bacculovirus, R&D
Systems) is only 15-18 kD. Using non-denaturating circum-
stances, the 21-28 kD substances form dimers so we believe the
material is mIL5, possibly in several differently glycosylated
forms. DNA vaccination results (see below) clearly support
20 this conclusion.

DNA vaccination of mice using murine IL5 AutoVac constructs:

A DNA vaccination study was performed in order to investigate
whether antibody responses specific for murine IL5 can be in-
25 duced by immunising mice with naked plasmid DNA encoding 8
different murine IL5 mutants. Since IL5 previously has been
reported to play a role in B cell differentiation, it is es-
sential to demonstrate that anti-mIL5 autoantibodies can be
generated in mice and B cell tolerance to mIL5 can be broken.
30

The general setup of the DNA vaccination experiments use ei-
ther C3H/Hen mice (H-2^k) or Balb/cA mice (H-2^d), 6-8 weeks old
divided into groups of 5 mice each. At days 0, 14, 28, 42, 62
and 76 the mice were anaestesized using hypnorm/dormicum s.c.

83

and injected with expression plasmids encoding ovalbumin (con-
trol), mIL5wt (wild type), or the mIL5 variants to be tested.
The DNA material was prepared using endofree GigaPrep kits
(Qiagen) and dissolved at 1 µg/ml in 0.15 M NaCl or 0.15 M

5   NaCl containing 0.1% bupivacaine. 100 µl material was injected
i.d. in each mouse at the lower back distributed at two injec-
tion sites. Prebleeds were obtained at day minus 2, and the
test bleedings were obtained at weeks 3, 5, 8 and 11. Sera
were isolated by centrifugation and stored at -20°C until

10 testing in ELISA for reactivity against purified ovalbumin and
mIL5 proteins.

A Typical result of a DNA vaccination experiment is shown in
Fig. 4. According to the general setup described above, 40

15 Balb/cA mice were immunized with ovalbumin control plasmid,
mIL5wt encoding plasmid or plasmids encoding the mIL5 AutoVac
variants mIL5.1 or mIL5.5. In this experiment, 9 out of 9 mice
immunized with ovalbumin encoding plasmid developed anti-oval-
bumin antibodies, whereas no anti-ovalbumin response was in-

20 duced in mice receiving the mIL5 wild type or mIL5 variant en-
coding DNA. Injection of mIL5wt encoding plasmid did not give
raise to an anti-mIL5 response, whereas the B cell tolerance
to mIL5 was broken in 4 out of 10 mice immunized with mIL5.1
plasmid and 7 out of 9 mice immunized with mIL5.5 encoding

25 plasmid DNA.

The main result of the whole series of DNA vaccination experi-
ments is summarized in the table below. The number of respon-
ders within an immunisation group differs between the diffe-

30 rent mIL5 AutoVac constructs and is dependent on the mouse
strain. Clearly, the mIL5.2 AutoVac construct is superior to
the other variants, being able to induce anti-mIL5 antibody
responses in both mouse strains with a penetrance of 100 %.

84

This plasmid (p820) also gave the highest expression levels in the COS transfection assay.

Another example to emphasize is the apparent MHC restriction

5 seen when using mIL5.4 encoding plasmid DNA as immunogen. Whereas only 1/10 C3H/Hen mice responds to the DNA vaccine, 9 out of 10 Balb/cA mice are responders. The opposite phenomenon (although not quite as pronounced) is seen with the mIL5.6 construct. The mIL5.2 DNA vaccine, however, seem to be

10 promiscuously immunogenic.

	OVAwt-pVax	mIL5wt-pcDNA	mIL5.1-pcDNA	mIL5.2-pcDNA	mIL5.4-pcDNA
Balb/cA	28/28	0/28	4/10	9/10	9/10
C3H/Hen	29/29	0/30	3/10	10/10	1/10

	mIL5.5-pcDNA	mIL5.6-pcDNA	mIL5.7-pcDNA	mIL5.12-pcDNA	mIL5.13-pcDNA
Balb/cA	7/9	0/10	2/10	0/10	0/10 *
C3H/Hen	5/10	6/10	2/10	2/10	2/10 *

15

Summary of the result of DNA vaccination of 280 mice. 6 mice died during the experiment for reasons not connected to the effects of the DNA vaccination. The number of responders (with high or intermediate anti-mIL5 titers) is shown in relation to the total number of mice within each immunization

20 group. *) bleedings obtained at day 55. All the other bleedings were obtained at day 77.

Another feature to mention is the tendency of mIL5 variants with the foreign T helper epitope inserted in mIL5 loop1 to be

25 stronger DNA vaccination immunogens than variants with the T helper epitope inserted in loop 3. This could be due to the relatively high expression levels. The only loop 2 variant tested, mIL5.4-pcDNA is only a strong immunogen in the Balb/cA strain, as mentioned above.

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85

Further characterization of the antibody responses induced by
DNA vaccination:

ELISA experiments were set up in order to determine whether
antibodies specific for the inserted T helper epitope could be
5 detected in anti-mIL5 positive mice. For each immunisation
group, sera from anti-mIL5 positive mice were pooled and
tested for reactivity against P2 or P30 peptides which had
been immobilised in AquaBind microtiter plates. Antisera in-
duced by DNA vaccination against mIL5.2 in both mouse strains
10 clearly contained reactivity against the inserted P30, whereas
none of the other antisera were reactive with P2 or P30. This
is probably connected to the higher antibody titers and pene-
trance that is generally observed with the mIL5.2 DNA vaccina-
tion construct. It should be mentioned that using this ELISA
15 setup we were able to detect anti-P2 reactivity in antisera
induced against mIL5.1.

The positive anti-mIL5 antiserum pools from the DNA vaccinated
mice were also tested in a competive ELISA for their ability
20 to inhibit the interaction between soluble native murine IL5
and monoclonal antibodies TRFK4 or TRFK5, which are both neu-
tralizing antibodies. Dilution series of anti-mIL5 antiserum
pools were preincubated with soluble native mIL5 and the sam-
ple was added to ELISA plates coated with catching antibody
25 TRFK5. Bound murine IL5 (which was not absorbed by the anti-
sera) was next visualised using layers of biotinylated TRFK4
and subsequently horse radish peroxidase labeled streptavidin.
Not all the anti-mIL5 positive antisera induced by DNA vacci-
nation could inhibit the interaction between soluble mIL5 and
30 TRFK4 or TRFK5. The antiserum with the highest TRFK4/5 inhi-
biting capability was from C3H/Hen mice immunized with mIL5.2
encoding DNA. It has not been tested whether the oberved dif-
ferences in inhibition is a direct measure of titer differ-
ences or it is connected to the fine specificity of the dif-

ferent antisera. Most likely, it is a combination of these two factors.

Animal model of eosinophilia in mIL5 AutoVac DNA immunized

mice:

40 DNA vaccinated mice were chosen for testing in an animal model of eosinophilia: 10 Balb/cA mice immunized with mIL5wt DNA, 10 Balb/cA mice immunized with mIL5.2 DNA, 10 C3H/Hen mice immunized with mIL5wt DNA and 10 C3H/Hen mice immunized with mIL5.2 DNA. A sensitization/challenging regimen with ovalbumin to induce eosinophilia was given to in each of these mice. The mice were sensitized with subcutaneous injections of 50 µg ovalbumin (OVA) in 0.9 % saline mixed 1:1 with Adjuphos once per week for three weeks. Four days after the last OVA sensitization the mice were challenged intranasally with 12.5 µg OVA in 0.9 % saline every other day for a total of 3 challenges. Bronchoalveolar lavage fluid (BALF) was collected one day after the last sensitization by cannulating the tracheae and washing the airway lumina with 1 ml PBS.

Approximately 30,000-60,000 BALF cells were spun unto slides at 1,500 rpm for 20 minutes. The slides were dried overnight and stained for 2.5 minutes with May-Grunwald stain (Sigma), washed for 4 minutes in tris buffered saline, stained for 20-30 minutes with Geimsa stain (1:20 with ddH2O; Sigma) and briefly rinsed with ddH2O. Stained slides were dried overnight and cell types were identified using light microscopy. Approximately 100-200 cells were counted per slide and 3 slides were counted per mouse. The eosinophil counts were expressed as the number of eosinophils per 100 cells counted. In mIL5.2 DNA vaccinated C3H/Hen mice, the induction of lung eosinophilia was significantly down-regulated compared to the wild type mIL5wt DNA vaccinated group (mIL5.2 DNA: 14.6 ± 8.9 eosinophils per 100 cells; mIL5wt DNA: 51.1 ± 9.9 eosinophils per

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100 cells). However, in the Balb/cA strain, there was no sig-
nificant difference in eosinophil counts between the immuniza-
tion groups (mIL5.2 DNA: 23.3 ± 6.8 eosinophils per 100 cells;
mIL5wt DNA: 27.7 ± 9.3 eosinophils per 100 cells). A possible

5 explanation is that Balb/cA mice are only weakly susceptible
to the model. This is supported by anti-ovalbumin ELISA data
showing that one week before the BALF collection the anti-
ovalbumin titers in serum from the Balb/cA mice were lower
than in serum from C3H/Hen. The Balb/cJ substrain is reported

10 to be susceptible to the OVA sensitization/challenge model.

EXAMPLE 20

Protein vaccination study

15

Balb/c J mice were immunized with murine IL5 (mIL5) protein
and subjected to an ovalbumin intranasal model that induces
eosinophils in the lungs of treated mice. Both the UniHis-mIL5
and the UniHis-mIL5.1 proteins induced antibodies that cross-

20 react with mIL-5 made in sf9 cells from R&D Systems. The
eosinophilia model induced high numbers of eosinophils in the
OVA control group and the UniHis-mIL5.1 groups, while the num-
bers of eosinophils were reduced in both the PBS group and the
UniHis-mIL5 group. This result led us to believe that the

25 groups may have been mixed.

Materials & Methods:

   UniHis-mIL-5   E1320 & E01397
30 UniHis-mIL-5.1   E01337 & E01396

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Immunizations:

6-8 week old female Balb/c J (M&B) mice were immunized with
either 1) nothing, 2) PBS, 3) UniHis-mIL5, or 4) UniHis-mIL-
5 5.1 in Complete Freund's Ajuvant (CFA; Sigma) and boosted 3
times at three week intervals with antigen in Incomplete
Freund's Adjuvant (IFA; Sigma). Sera was collected and tested
in an ELISA 10 days after each boost.

10 ELISAs:

Anti-UniHis-mIL5 ELISA:
Sera were obtained at days 32 (bleed 1) and 54 (bleed 2) after
2 and 3 immunizations, respectively. Polystyrene microtiter
15 plates (Maxisorp, Nunc) were coated with purified HIS-mIL5wt
(0.1 µg/well, E1320). The reactivities of diluted sera added
to the wells were visualised using a goat anti-mouse secondary
antibody. OD490 readings of control sera from mice immunized
with PBS in Freunds adjuvans were subtracted from the OD490
20 readings of the test samples.

Anti-mIL5 ELISA:
Sera were obtained at day 75 (bleed 3). Polystyrene microtiter
plates (Maxisorp, Nunc) were coated with purchased mIL5 (0.1
25 µg/well, R&D cat. no. 405-ML). The reactivities of 1:1000 di-
luted sera added to the wells were visualised using a goat
anti-mouse secondary antibody. The reactivity of TRFK5 (2
µg/ml) was visualised using a rabbit anti-rat secondary anti-
body.
30
Competitive ELISA:
Dilutions of antisera were preincubated with soluble IL5 for 1
hour and added to polystyrene microtiter plates (Maxisorp,
Nunc) which were coated with catching antibody TRFK5. Bound

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mIL5 was visualised using biotinylated TRFK4 and a HRP la-
belled goat anti-mouse secondary antibody.

Anti-P2 ELISA:

5   Pools of antisera from HIS-mIL5wt, HIS-mIL5.1 or PBS immunised
    mice were tested for reactivity against P2 peptide in ELISA.
    Specialized microtiter plates (Aquabind, M&E Biotech) were
    coated with 0.5 µg/well synthetic P2 peptide. The reactivities
    of diluted sera added to the wells were visualised using a HRP
10 labelled goat anti-mouse secondary antibody (1:2000, Dako).

Anti-UniHis ELISA:

    Pools of antisera from HIS-mIL5wt, HIS-mIL5.1 or PBS immunised
    mice were tested for reactivity against HIS-tag peptide
15 (UNIZYME) in ELISA. Specialized microtiter plates (AquaBind,
    M&E Biotech) were coated with 0.5 µg/well synthetic HIS-tag
    peptide. The reactivities of diluted sera added to the wells
    were visualised using a HRP labelled goat anti-mouse secondary
    antibody (1:2000, Dako).

20

Anti-S2 background protein ELISA:

    Pools of antisera from HIS-mIL5wt, HIS-mIL5.1 or PBS immunised
    mice were tested for reactivity against S2 background prepara-
    tion in ELISA. Polystyrene microtiter plates (Maxisorp, Nunc)
25 were coated with 0.1 µg/well S2 background preparation. The
    reactivities of diluted sera added to the wells were visua-
    lised using a HRP labelled goat anti-mouse secondary antibody
    (1:2000, Dako).

30 Anti-BSA ELISA:

    Pools of antisera from HIS-mIL5wt, HIS-mIL5.1 or PBS immunised
    mice were tested for reactivity against BSA in ELISA. Polysty-
    rene microtiter plates (Maxisorp, Nunc) were coated with 10
    µg/well BSA (Intergen). The reactivities of diluted sera added

to the wells were visualised using a HRP labelled goat anti-
mouse secondary antibody (1:2000, Dako).

Eosinophilia Model:

5 Balb/c J mice were sensitized with subcutaneous injections of
50 μg ovalbumin (OVA) in 0.9% saline mixed 1:1 with Adjuphos
as alum adjuvant. OVA immunizations were repeated once per
week for four weeks. One week after the last OVA sensitiza-
tion, the mice were challenged with 12.5 μg OVA in 0.9% saline
10 intranasal every other day for a total of 3 challenges. Bron-
choalveolar lavage fluid (BALF) was collected one day after
the last sensitization by cannulating the tracheae and washing
the airway lumina with 1 ml 0.9% saline, or PBS.

15 BAL staining:

Approximately 30,000-60,000 BALF cells were spun unto slides
at 1,500 rpm for 20 minutes. The slides were dried overnight
and stained for 2.5 minutes with May-Grunwald stain (Sigma),
washed for 4 minutes in TBS, stained for 20-30 minutes with
20 Giemsa stain (1:20 with ddH_2O; Sigma) and briefly rinsed with
ddH_2O. Stained slides were dried overnight and cell types were
identified using light microscopy. Approximately 100-200 cells
were counted per slide and 3 slides were counted per mouse.

25 **Results:**

Detection of anti-mIL5 antibodies:

A series of ELISA experiments were performed in order to in-
vestigate whether antibody responses specific for murine IL5
30 were induced in mice immunized with HIS-mIL5wt and HIS-mIL5.1
protein material. First, it was determined if antibodies
against the HIS-mIL5wt immunization material were elicited by
testing dilutions of antisera from individual mice on ELISA
plates coated with the HIS-mIL5wt material. It was found that

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already by bleed one, all mice had developed high-titered an-
tibody responses against the HIS-mIL5wt material (E1320, ex-
pressed from Drosophila S2 cells and purified) which was esti-
mated to be approximately 95% pure.

5

This result is not a firm confirmation that the antisera
cross-reacts with murine IL5. In this setup, reactivities
would also be detected against impurities from the Drosophila
S2 cells, the S2 medium (which contain e.g. BSA from fetal
10 calf serum, the HIS-tag as well as denatured mIL5 B cell epi-
topes. To demonstrate, that the antibodies induced contain re-
activities against native murine IL5, the sera were tested in
ELISA plates coated with mIL5 purchased from R&D systems. This
material (R&D cat. no. 405-ML) is biologically active, con-
15 tains no HIS-tag, is expressed in the bacculovirus Sf21 sys-
tem, is also very pure (97 %), and can be purchased free of
carrier-protein (BSA). Pooled sera from both immunisation
groups reacted with the purchased mIL5 coated on ELISA plates,
whereas sera from PBS immunised mice did not. This was shown
20 when testing sera from bleed 3 obtained at day 75, 11 days af-
ter the 4th immunization, but also sera from bleed 1 and 2 re-
acts with the purchased mIL5 in a similar setup. In order to
exclude signals from cross-reaction with the BSA carrier, the
experiments were repeated for bleeds 1 and 2 using carrier-
25 free versions of the purchased mIL5 material and BSA-free
ELISA buffers, and still high anti-mIL5 responses are seen.

To further confirm that the induced antisera cross-react with
native mIL5, a competitive ELISA was set up. This ELISA tests
30 the ability of the different antisera to inhibit the interac-
tion between soluble native murine IL5 and monoclonal antibo-
dies TRFK4 or TRFK5, which are both neutralizing antibodies.
Dilution series of antiserum pools were preincubated with
soluble native mIL5 and the samples were added to ELISA plates

coated with catching antibody TRFK5. Bound murine IL5 (which
was not absorbed by the antisera) was next visualised using
layers of biotinylated TRFK4 and subsequently horseradish per-
oxidase labeled streptavidin. An anti-mIL5 positive and an

5   anti-mIL5 negative antiserum from DNA vaccinated mice were in-
cluded as controls. It was demonstrated that antisera from
both HIS-mIL5wt and HIS-mIL5.1 immunized mice could inhibit
the interaction between soluble mIL5 and TRFK4 or TRFK5.

10 Based on the above-referenced it is concluded that mIL5 spe-
cific autoantibodies are induced in mice immunized with either
the HIS-mIL5wt or the HIS-mIL5.1 protein preparations (in 100%
of the mice tested). In other words, B cell tolerance to mIL5
can be broken using recombinant HIS-tagged versions of both

15 wild type and AutoVac murine IL5. A plausible explanation for
the observation that B cell tolerance is broken to the wild
type protein is that the HIS-tag in these mice functions as a
"foreign" immunogenic T helper epitope. Another explanation
could be that the administration of Complete Freund's Adjuvant

20 breaks B cell tolerance to mIL5. These hypotheses can be
tested using non-HIS tagged antigens and/or alternative, less
strong adjuvants such as AdjuPhos.

Further characterization of the antibody responses in mice im-
25 munized with mIL5 AutoVac proteins:
ELISA experiments were set up in order to determine whether
antibodies specific for the inserted T helper epitope could be
detected in sera from mIL5 protein immunised mice. For each
immunisation group, antisera (bleed 2) were pooled and tested

30 for reactivity against synthetic P2 peptide which had been im-
mobilised in AquaBind microtiter plates. Anti-HIS-mIL5.1 an-
tiserum contained reactivity against the inserted P2 peptide,
whereas neither anti-HIS-mIL5wt or anti-PBS/CFA reacted with
the peptide.

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It was also tested whether the the anti-HIS-mILwt and anti-
HIS-mIL5.1 antisera contained reactivity against the 15-mer
HIS-tag (UNIZYME HIS-tag, SEQ ID NO: 25) that is fused to the
5 N-terminal of both the wild type and AutoVac mIL5 proteins.
The peptide was synthesized and covalently immobilized in
AquaBind microtiter plates, and pooled antisera from each im-
munization group (bleeds 1, 2 and 3) were tested for reacti-
vity against the bound peptide. Antisera from all protein im-
10 munized mice reacted with the synthetic HIS-tag peptide.

It was also tested whether the anti-HIS-mIL5wt and anti-HIS-
mIL5.1 antisera was reactive with components from the S2 Dro-
sophila cells or culture medium. ELISA plates coated with BSA
15 (a major medium component) or S2-background preparation (gen-
erated by subjecting culture supernatant from Her2 expressing
Drosophila S2 cells to a purification scheme similar to that
of the mIL5 purification procedure). The results of these
analyses demonstrated that whereas the anti-BSA responses were
20 very low, the reactions with the S2-background material were
pronounced.

Eosinophil Counts in BALF:
To determine if the anti-IL5 antibodies in vaccinated mice
25 could down-regulate the in vivo activity of IL5, we induced
IL5-dependent eosinophilia in the lungs of the vaccinated
mice. Eosinophils were induced by challenging sensitized mice
with OVA intranasally. High numbers of eosinophils were in-
duced in control OVA mice and mice vaccinated with UniHis-
30 mIL5.1, but not in Uni-His-mIL5 or PBS vaccinated mice. The
discrepancy of eosinophil numbers between control groups (OVA
and PBS) and experimental groups (UniHis-mIL5 and UniHis-
mIL5.1), and the positive results from the DNA vaccinated mice
reported above, led us to believe that the groups may have

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been switched. However, no attempts to demonstrate a switch supported this interpretation. The protein vaccinations are being repeated in an identical setup to clarify this controversy.

5

Discussion:

The ability of both the UniHis-mIL5 and UniHis-mIL5.1 proteins to induce antibodies that cross-react with wildtype murine IL5

10 was clearly demonstrated. Whether the ability of the UniHis-mIL5 protein to bypass immunological tolerance is due to the UniHis-tag, or some other reason (*e.g.* CFA adjuvant) remains to be clarified. It was surprising to see that only the UniHis-mIL5 construct was able to down-regulate the endogenous *in*

15 *vivo* activity of mIL5 in an eosinophilia model. This inability of antisera generated from UniHis-mIL5.1 protein vaccination to inhibit eosinophilia, and its ability to inhibit eosinophilia via DNA vaccinations suggests that a technical mistake may have occurred in this experiment. This is also supported

20 by the unusual finding of PBS vaccination inhibiting eosinophilia. This most likely explanation is that these two groups (PBS and UniHis-mIL5.1) were switched.

25 **LIST OF REFERENCES**

Akutsu I. *et al.* ,1995, Immunol. Lett., **45**: 109-116.
Alexander A.G. *et al.*, 1994, Thorax, **49**(12): 1231-1233.

30 Azuma C. *et al.*, 1986, Nucleic Acid Res. 1986, **14**(22): 9149-9158.

Barata L.T. *et al.*, 1998, J. Allergy and Clin. Immunol, **101**: 222-230.
35
Baumann M.A. *et al.*, 1997, Methods, **11**: 88-97

95

Callard R.E. & Gearing A.J.H., 'IL-5', Cytokine Facts Book 1994, Academic Press.

Campbell H.D. *et al.*, 1988, Eur. J. Biochem., **174**: 345-352.

Chand N. *et al.*, 1992, Eur. J. Immunol., **211**: 121-123.

Coeffier E. *et al.*, 1994, Br. J. Pharmacol., **113**(3): 749-56.

Coffman R.L. *et al.*, 1989, Science, **245**: 308-310.

Corrigan C.J. & Kay A.B., 1996, Eur. Resp. J., **9**, suppl. 22: 72s-78s.

Cousins D.J. *et al.*, 1994, Am. J. Resp. Crit. Care. Med., **150**: S50-S53.

Danzig M. *et al.*, 1997, Allergy, **52**(8): 787-794.

Dickason R.R. *et al.*, 1994, Cytokine, **6**(6): 647-656.

Dickason R.R. *et al.*, 1996a, Nature, **379**: 652-655.

Dickason R.R. *et al.*, 1996b, J. Mol. Med., **74**(9), 535-546

Egan R.W. *et al.*, 1995, Int. Arch. Allergy Immunol., **107**: 321-322.

Foster P.S. *et al.*, 1996, J. Exp. Med., **183**: 195-201.

Graber P. *et al.*, 1993, Eur. J. Biochem., **212**(3): 751-755.

Graber P. *et al.*, 1995, J. Biol. Chem., **270**(26): 15762-15769.

Hamelmann E. *et al.*, 1997, Am. J. Crit. Care Med., **155**(3): 819-825.

Huston M.M. *et al.*, 1996, J. Immunol., **156**(4): 1392-1401.

Karlen S. *et al.*, 1998, Int. Rev. Immunol., **16**(3-4): 227-247.

Kodama S. *et al.*, 1993, Eur. J. Biochem., **211**(3): 903-908.

Kopf M. *et al.*, 1996, Immunity, **4**: 15-24.

Kung T.T. *et al.*, 1995, Am. J. Respir. Cell. Mol. Biol., **13**: 360-365.

Lee N.A. *et al.*, 1997a, J. Immunol., **158**: 1332-1344.

96

Lee J.J. *et al.*, 1997b, J. Exp. Med. 1997b, **185**(12): 2143-2156.

Lopez A.F. *et al.*, 1992, Immunology Today, **13**: 495-500.

Mauser P.J. *et al.*, 1993, Am. Rev. Respir. Dis., **148**: 1623-1627.

Mauser P.J. *et al.*, 1995, Am. J. Respir. Crit. Care Med., **152**(2): 467-472.

Milburn M.V. *et al.*, 1993, Nature, **363**: 172-176.

Mori A. *et al.*, 1997, J. Allergy Clin. Immunol., **100**(6) Pt 2: S56-64.

Moxham J. & Costello J.F., 'Respiratory diseases', chapt. 14, Textbook of Medicine, Churchill Livingstone 1990, Ed. Souhami R.L. and Moxham J.

Nagai H. *et al.*, 1993a, Ann. N.Y. Acad.Sci., 91-96.

Nagai H. *et al.*, 1993b, Life Sciences, **53**: PL 243-247.

Ohashi Y. *et al.*, 1998, Scand. J. Immunol, **47**: 596-602.

Ortega D. & Busse W.W., 'Asthma: Pathogenesis and treatment', chapt. 28, Allergy, W.B. Saunders Company 1997, Ed. Kaplan A.P.

Proudfoot A.E. *et al.*, 1990, Biochem J., **270**(2): 357-361.

Proudfoot A.E. *et al.*, 1996, J. Protein Chem., **15**(5): 491-499.

Rose K. *et al.*, 1992, Biochem J, **286**(Pt 3): 825-828.

Sanderson C.J., 1992, Blood, **79**(12): 3101-3109.

Sher A. *et al.*, 1990, J. Immunol., **145**: 3911-3916.

Takatsu K. *et al.*, Interleukin-5, Growth Factors and Cytokines in Health and Disease 1997, vol.2A, JAI Press Inc., Ed. Leroith D. & Bondy C.

Tanabe T. *et al.*, 1987, J. Biol. Chem., **262**: 16580-16584.

Tavernier J. *et al.*, 1989, DNA, **8**(7), 491-501.

Tominaga A. *et al.*, 1990, J. Immunol., **144**(4): 1345-1352.

Tominaga A. *et al.*, 1991, J. Exp. Med., **173**(2): 429-437.

Underwood D.C. *et al.*, 1996, Am. J. Resp. Crit. Care Med., **154**: 850-857.

5 van Oosterhout A.J.M. *et al.*, 1993, Am. Rev. Resp. Dis., **147**: 548-552.

van Oosterhout A.J.M. *et al.*, 1995, Am. J. Respir. Crit. Care Med., **151**: 177-183.

10

Villinger F. *et al.*, 1995, J. Immunol., **155**: 3946-3954.

Wang P. *et al.*, 1998, J. Immunol., **160**: 4427-4432.

15 Yamaguchi Y. *et al.*, 1991, Blood, **78**(10): 2542-2547.

CLAIMS

1. A method for *in vivo* down-regulation of interleukin 5 (IL5)
activity in an animal, including a human being, the method
5 comprising effecting presentation to the animal's immune sys-
tem of an immunogenically effective amount of

   - at least one IL5 polypeptide or subsequence thereof which
     has been formulated so that immunization of the animal
     with the IL5 polypeptide or subsequence thereof induces
10   production of antibodies against the IL5 polypeptide,
     and/or
   - at least one IL5 analogue wherein is introduced at least
     one modification in the IL5 amino acid sequence which has
     as a result that immunization of the animal with the ana-
15   logue induces production of antibodies against the IL5
     polypeptide.

2. The method according to claim 1, wherein is presented an
IL5 analogue with at least one modification of the IL5 amino
20 acid sequence.

3. The method according to claim 2, wherein the modification
has as a result that a substantial fraction of IL5 B-cell epi-
topes are preserved and that
25 -   at least one foreign T helper lymphocyte epitope (T_H epi-
        tope) is introduced, and/or
   -    at least one first moiety is introduced which effects
        targeting of the modified molecule to an antigen present-
        ing cell (APC) or a B-lymphocyte, and/or
30 -   at least one second moiety is introduced which stimulates
        the immune system, and/or
   -    at least one third moiety is introduced which optimizes
        presentation of the modified IL5 polypeptide to the im-
        mune system.

35
4. The method according to claim 3, wherein the modification
includes introduction as side groups, by covalent or non-cova-
lent binding to suitable chemical groups in IL5 or a subse-

quence thereof, of the foreign T_H epitope and/or of the first and/or of the second and/or of the third moiety.

5. The method according to claim 3 or 4, wherein the modification includes amino acid substitution and/or deletion and/or insertion and/or addition.

6. The method according to claim 5, wherein the modification results in the provision of a fusion polypeptide.

7. The method according to claim 5 or 6, wherein introduction of the amino acid substitution and/or deletion and/or insertion and/or addition results in a substantial preservation of the overall tertiary structure of IL5.

8. The method according to any one of claims 2-7, wherein the modification includes duplication of at least one IL5 B-cell epitope and/or introduction of a hapten.

9. The method according to any one of claims 3-8, wherein the foreign T-cell epitope is immunodominant in the animal.

10. The method according to any one of claims 3-9, wherein the foreign T-cell epitope is promiscuous.

11. The method according to claim 10, wherein the at least one foreign T-cell epitope is selected from a natural promiscuous T-cell epitope and an artificial MHC-II binding peptide sequence.

12. The method according to claim 11, wherein the natural T-cell epitope is selected from a Tetanus toxoid epitope such as P2 or P30, a diphtheria toxoid epitope, an influenza virus hemagluttinin epitope, and a *P. falciparum* CS epitope.

13. The method according to any one of claims 3-12, wherein the first moiety is a substantially specific binding partner for a B-lymphocyte specific surface antigen or for an APC spe-

cific surface antigen, such as a hapten or a carbohydrate for
which there is a receptor on the B-lymphocyte or the APC.

14. The method according to any one of claims 3-13, wherein
5 the second moiety is selected from a cytokine, a hormone, and
a heat-shock protein.

15. The method according to claim 6, wherein the cytokine is
selected from, or is an effective part of, interferon γ (IFN-
10 γ), Flt3L, interleukin 1 (IL-1), interleukin 2 (IL-2), inter-
leukin 4 (IL-4), interleukin 6 (IL-6), interleukin 12 (IL-12),
interleukin 13 (IL-13), interleukin 15 (IL-15), and granulo-
cyte-macrophage colony stimulating factor (GM-CSF), and the
heat-shock protein is selected from, or is an effective part
15 of any of, HSP70, HSP90, HSC70, GRP94, and calreticulin (CRT).

16. The method according to any one of claims 3-15, wherein
the third moiety is of lipid nature, such as a palmitoyl
group, a myristyl group, a farnesyl group, a geranyl-geranyl
20 group, a GPI-anchor, and an N-acyl diglyceride group.

17. The method according to any one of the preceding claims,
wherein the IL5 polypeptide has been modified in at least one
of loops 1-3 or in the amino acid residues C-terminal to helix
25 D, said loops and said helix D corresponding to those shown in
Fig. 3 for human and murine IL5.

18. The method according to claim 17, wherein the IL5 polypep-
tide is a human IL5 polypeptide.
30
19. The method according to claim 18, wherein the human IL5
polypeptide has been modified by substituting at least one
amino acid sequence in SEQ ID NO: 1 with at least one amino
acid sequence of equal or different length thereby giving rise
35 to a foreign T$_H$ epitope, wherein substituted amino acid resi-
dues are selected from the group consisting of residues 87-90,
residues 88-91, residues 32-43, residues 33-43, residues 59-
64, residues 86-91, and residues 110-113.

20. The method according to any one of the preceding claims, wherein presentation to the immune system is effected by having at least two copies of the IL5 polypeptide, the subse-

5 quence thereof or the modified IL5 polypeptide covalently of non-covalently linked to a carrier molecule capable of effecting presentation of multiple copies of antigenic determinants.

10 21. The method according to any the preceding claims, wherein the IL5 polypeptide, the subsequence thereof, or the modified IL5 polypeptide has been formulated with an adjuvant which facilitates breaking of autotolerance to autoantigens.

15 22. The method according to claim 21, wherein the adjuvant is selected from the group consisting of an immune targeting adjuvant; an immune modulating adjuvant such as a toxin, a cytokine and a mycobacterial derivative; an oil formulation; a polymer; a micelle forming adjuvant; a saponin; an immu-

20 nostimulating complex matrix (an ISCOM matrix); a particle; DDA; aluminium adjuvants; DNA adjuvants; γ-inulin; and an encapsulating adjuvant.

23. The method according to any one of the preceding claims,

25 wherein an effective amount of the IL5 polypeptide or the IL5 analogue is administered to the animal via a route selected from the parenteral route such as the intradermal, the subdermal, the intracutaneous, the subcutaneous, and the intramuscular routes; the peritoneal route; the oral route; the buccal

30 route; the sublinqual route; the epidural route; the spinal route; the anal route; and the intracranial route.

24. The method according to claim 23, wherein the effective amount is between 0.5 µg and 2,000 µg of the IL5 polypeptide,

35 the subsequence thereof or the analogue thereof.

25. The method according to claim 23 or 24, which includes at least one administration of the IL5 polypeptide or analogue

per year, such as at least 2, at least 3, at least 4, at least 6, and at least 12 administrations per year.

26. The method according to any one of claims 23-25, wherein
5 the IL5 polypeptide or analogue is contained in a virtual lymph node (VLN) device.

27. The method according to any one of claims 1-20, wherein presentation of modified IL5 to the immune system is effected
10 by introducing nucleic acid(s) encoding the modified IL5 into the animal's cells and thereby obtaining *in vivo* expression by the cells of the nucleic acid(s) introduced.

28. The method according to claim 27, wherein the nucleic
15 acid(s) introduced is/are selected from naked DNA, DNA formulated with charged or uncharged lipids, DNA formulated in liposomes, DNA included in a viral vector, DNA formulated with a transfection-facilitating protein or polypeptide, DNA formulated with a targeting protein or polypeptide, DNA formulated
20 with Calcium precipitating agents, DNA coupled to an inert carrier molecule, DNA encapsulated in chitin or chitosan, and DNA formulated with an adjuvant such as the adjuvants defined in claim 22.

25 29. The method according to claim 27 or 28, wherein the nucleic acids are administered intraarterially, intraveneously, or by the routes defined in claim 23.

30. The method according to claim 28 or 29, wherein the nu-
30 cleic acid(s) is/are contained in a VLN device.

31. The method according to any one of claims 28-30, which includes at least one administration of the nucleic acids per year, such as at least 2, at least 3, at least 4, at least 6,
35 and at least 12 administrations per year

32. A method for treating and/or preventing and/or ameliorating asthma or other chronic allergic conditions characterized

by eosinophilia, the method comprising down-regulating IL5 activity according to the method of any one of claims 1-31 to such an extent that the number of eosinophil cells, either systemically or locally at the disease focus, is significantly

5 reduced, such as a reduction of at least 20%.

33. An IL5 analogue which is derived from an animal IL5 polypeptide wherein is introduced a modification which has as a result that immunization of the animal with the analogue in-

10 duces production of antibodies against the IL5 polypeptide.

34. An IL5 analogue according to claim 33, wherein the modification is as defined in any one of claims 1-22.

15 35. An immunogenic composition comprising an immunogenically effective amount of an IL5 polypeptide autologous in an animal, said IL5 polypeptide being formulated together with an immunologically acceptable adjuvant so as to break the animal's autotolerance towards the IL5 polypeptide, the composi-

20 tion further comprising a pharmaceutically and immunologically acceptable carrier and/or vehicle.

36. An immunogenic composition comprising an immunogenically effective amount of an IL5 analogue according to claim 33 or

25 34, the composition further comprising a pharmaceutically and immunologically acceptable carrier and/or vehicle and optionally an adjuvant.

37. An immunogenic composition according to Claim 35 or 36,

30 wherein the adjuvant is selected from the group consisting of the adjuvants of claim 22.

38. A nucleic acid fragment which encodes an IL5 analogue according to claim 33 or 34.

35

39. A vector carrying the nucleic acid fragment according to claim 38.

40. The vector according to claim 39 which is capable of autonomous replication.

41. The vector according to claim 39 or 40 which is selected from the group consisting of a plasmid, a phage, a cosmid, a mini-chromosome, and a virus.

42. The vector according to any one of claims 39-41, comprising, in the 5'→3' direction and in operable linkage, a promoter for driving expression of the nucleic acid fragment according to claim 38, optionally a nucleic acid sequence encoding a leader peptide enabling secretion of or integration into the membrane of the polypeptide fragment, the nucleic acid fragment according to claim 38, and optionally a terminator.

43. The vector according to any one of claims 39-42 which, when introduced into a host cell, is integrated in the host cell genome.

44. The vector according to any one of claims 39-42 which, when introduced into a host cell, is not capable of being integrated in the host cell genome.

45. The vector according to any one of claims 39-44, wherein the promoter drives expression in a eukaryotic cell and/or in a prokaryotic cell.

46. A transformed cell carrying the vector of any one of claims 39-45.

47. The transformed cell according to claim 46 which is capable of replicating the nucleic acid fragment according to claim 38.

48. The transformed cell according to claim 47, which is a microorganism selected from a bacterium, a yeast, a protozoan, or a cell derived from a multicellular organism selected from

a fungus, an insect cell such as an S_2 or an SF cell, a plant cell, and a mammalian cell.

49. The transformed cell according to claim 48 which is a bacterium of the genus *Escherichia*, *Bacillus*, *Salmonella*, or *Mycobacterium*.

50. The transformed cell according to claim 52, which is selected from the group consisting of an *E. coli* cell, and a non-pathogenic *Mycobacterium* cell such as *M. bovis* BCG.

51. The transformed cell according to any one of claims 46-50, which expresses the nucleic acid fragment according to claim 38.

52. The transformed cell according to claim 55, which secretes or carries on its surface, the IL5 analogue according to claim 33 or 34.

53. The method according to any one of claims 1-20, wherein presentation to the immune system is effected by administering a non-pathogenic microorganism or virus which is carrying a nucleic acid fragment which encodes and expresses the IL5 polypeptide or analogue.

54. The method according to claim 53, wherein the virus is a non-virulent pox virus such as a vaccinia virus.

55. The method according to claim 54, wherein the microorganism is a bacterium, such as a bacterium defined in claim 49 or 50.

56. The method according to any one of claims 53-55, wherein the non-pathogenic microorganism or virus is administered one single time to the animal.

57. A composition for inducing production of antibodies against IL5, the composition comprising

- a nucleic acid fragment according to claim 38 or a vector according to any one of claims 39-45, and

- a pharmaceutically and immunologically acceptable carrier and/or vehicle and/or adjuvant.

58. The composition according to claim 57, wherein the nucleic acid fragment is formulated according to claim 28 or 30.

59. A stable cell line which carries the vector according to any one of claims 39-45 and which expresses the nucleic acid fragment according to claim 38, and which optionally secretes or carries the IL5 analogue according to claim 33 or 34 on its surface.

60. A method for the preparation of the cell according to any one of claims 46-52, the method comprising transforming a host cell with the nucleic acid fragment according to claim 38 or with the vector according to any one of claims 39-45.

61. A method for the identification of a modified IL5 polypeptide which is capable of inducing antibodies against unmodified IL5 in an animal species where the unmodified IL5 polypeptide is a self-protein, the method comprising

- preparing, by means of peptide synthesis or genetic engineering techniques, a set of mutually distinct modified IL5 polypeptides wherein amino acids have been added to, inserted in, deleted from, or substituted into the amino acid sequence of an IL5 polypeptide of the animal species thereby giving rise to amino acid sequences in the set which comprise T-cell epitopes which are foreign to the animal species, or preparing a set of nucleic acid fragments encoding the set of mutually distinct modified IL5 polypeptides,

- testing members of the set for their ability to induce production of antibodies by the animal species against the unmodified IL5, and

-       identifying and optionally isolating the member(s) of the
        set which significantly induces antibody production
        against unmodified IL5 in the animal species, or identi-
        fying and optionally isolating the polypeptide expression
5   products encoded by embers of the set of nucleic acid
        fragments which significantly induces antibody production
        against unmodified IL5 in the animal species.

62. A method for the preparation of an immunogenic composition
10 comprising at least one modified IL5 polypeptide which is ca-
   pable of inducing antibodies against unmodified IL5 in an ani-
   mal species where the unmodified IL5 polypeptide is a self-
   protein, the method comprising

15 -   preparing, by means of peptide synthesis or genetic engi-
        neering techniques, a set of mutually distinct modified
        IL5 polypeptides wherein amino acids have been added to,
        inserted in, deleted from, or substituted into the amino
        acid sequence of an IL5 polypeptide of the animal species
20   thereby giving rise to amino acid sequences in the set
        comprising T-cell epitopes which are foreign to the ani-
        mal,
   -    testing members of the set for their ability to induce
        production of antibodies by the animal species against
25   the unmodified IL5, and
   -    admixing the member(s) of the set which significantly
        induces production of antibodies in the animal species
        which are reactive with IL5 with a pharmaceutically and
        immunologically acceptable carrier and/or vehicle, op-
30   tionally in combination with at least one pharmaceuti-
        cally and immunologically acceptable adjuvant.

63. The method according to claim 61 or 62, wherein prepara-
   tion of the members of the set comprises preparation of mutu-
35 ally distinct nucleic acid sequences, each sequence being a
   nucleic acid sequence according to claim 38, insertion of the
   nucleic acid sequences into appropriate expression vectors,
   transformation of suitable host cells with the vectors, and

expression of the nucleic acid sequences, optionally followed by isolation of the expression products.

64. The method according to claim 63, wherein the preparation
5 of the nucleic acid sequences and/or the vectors is achieved by the aid of a molecular amplification technique such as PCR, or by the aid of nucleic acid synthesis.

65. Use of IL5 or a subsequence thereof for the preparation of
10 an immunogenic composition comprising an adjuvant for down-regulating IL5 activity in an animal.

66. Use of IL5 or a subsequence thereof for the preparation of an immunogenic composition comprising an adjuvant for the
15 treatment, prophylaxis or amelioration of asthma or other chronic allergic conditions.

67. Use of an IL5 analogue for the preparation of an immuno-genic composition optionally comprising an adjuvant for down-
20 regulating IL5 activity in an animal.

68. Use of an IL5 analogue for the preparation of an immuno-genic composition optionally comprising an adjuvant for the treatment, prophylaxis or amelioration of asthma or other
25 chronic allergic conditions.

```
                                        10                                        20
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 *    *   Met               Met                Thr Val                     Thr Gln   Ala

                                        30                                        40
His-Arg-Thr-Leu-Leu-Ile-Ala-Asn-Glu-Thr-Leu-Arg-Ile-Pro-Val-His-Lys-Asn-
              Ala               Thr Ser     Met Leu                          Thr

                                        50                                        60
His-Gln-Leu-Cys-Thr-Glu-Glu-Ile-Phe-Gln-Gly-Ile-Gly-Thr-Leu-Glu-Ser-Gln-Thr-Val-
                    Ile Gly               Leu Asp Ile               Lys Asn

                                        70                                        80
Gln-Gly-Gly-Thr-Val-Glu-Arg-Leu-Phe-Lys-Asn-Leu-Ser-Leu-Ile-Lys-Lys-Tyr-Ile-Asp-
Arg               Met                      Gln

                                        90                                       100
Gly-Gln-Lys-Lys-Lys-Cys-Gly-Glu-Glu-Arg-Arg-Arg-Val-Asn-Gln-Phe-Leu-Asp-Tyr-Leu-
Arg               Glu                          Thr Arg

                                       110                                       115
Gln-Glu-Phe-Leu-Gly-Val-Met-Asn-Thr-Glu-Trp-Ile-Ile-Glu-Ser
              Ser                    Ala Met     Gly
```

Fig. 1

Fig. 2A

NH

NH'

Fig. 2B

Fig. 2C

hIL-5   IPTEIPTSALVKETLALLSTHRTLLIANETLRIPVPVHKNHQLCTEEIFQGIGTLES   57
         * *   -*-**-*-*---*-*-*---*---*--*-*-*---**-* -* -**
mIL-5   MEIPMSTVVKETLTQLSAHRALLTSNETMRLPVPTHKNHQLCIGEIFQGLDILKN   55

              Helix A   Loop 1   Helix B

hIL-5   QTVQGGTVERLFKNLSLIKKYIDGQKKKCGEERRRVNQFLDYLQEFLGVMNTEWIIES   115
         -*-* ---*-*-*-*-------*--*-* *-* --** ------------* * -
mIL-5   QTVRGGTVEMLFQNLSLIKKYIDRQKEKCGEERRRTRQFLDYLQEFLGVMSTEWAMEG   113

              Helix C   Loop 3   Helix D

         Loop 2

Fig. 3

Fig. 4

Fig. 5

1

SEQUENCE LISTING

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      Klysner, Steen

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Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
            35   40   45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50   55   60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
65   70   75   80

Gly Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val Asn Gln Phe
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Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
            35   40   45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50   55   60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
65   70   75   80

Gly Gln Lys Lys Lys Cys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile
                85   90   95

Gly Ile Thr Glu Leu Arg Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln
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Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Cys Thr
        35                  40                  45

Glu Glu Ile Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln
    50                  55                  60

Gly Gly Thr Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys
65                  70                  75                  80

Tyr Ile Asp Gly Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val
                85                  90                  95

Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr
                100                 105                 110

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              20                  25                  30


Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
         35                  40                  45


Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Gln Tyr Ile Lys Ala Asn
     50                  55                  60


Ser Lys Phe Ile Gly Ile Thr Glu Leu Val Glu Arg Leu Phe Lys Asn
 65              70                  75                  80


Leu Ser Leu Ile Lys Lys Tyr Ile Asp Gly Gln Lys Lys Lys Cys Gly
                85                  90                  95


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            20              ·           25              30

Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
            35              40              45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
        50              55              60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
    65              70              75              80

Gly Gln Lys Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
            85              90              95

Ile Thr Glu Leu Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe
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 65              70              75              80

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Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Gln Tyr Ile
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     50              55              60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
 65              70              75              80

Gly Gln Lys Lys Lys Cys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu
            85              90              95
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        35   40   45

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Glu Ser Gln Thr Val Gln Gly Gly Thr Val Glu Arg Leu Phe Lys Asn
65   70   75   80

Leu Ser Leu Ile Lys Lys Tyr Ile Asp Gly Gln Lys Lys Lys Cys Gly
                85   90   95

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<210> 9
<211> 130
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Human IL5
      modified by substitution with tetanus toxoid P30
      epitope

<220>
<221> MUTAGEN
<222> (59)..(79)
<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(58)
<223> Identical to residues 1-58 in SEQ ID NO: 1

<220>
<221> SIMILAR
<222> (80)..(130)
<223> Identical to residues 65-115 in SEQ ID NO: 1

<400> 9
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
  1               5                  10                  15

Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
             20                  25                  30

Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
         35                  40                  45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Phe Asn Asn Phe Thr Val
     50                  55                  60

Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Val
 65                  70                  75                  80

Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Gly
                 85                  90                  95

Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val Asn Gln Phe Leu
                100                 105                 110

Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Glu Trp Ile Ile
         115                 120                 125

Glu Ser
    130


<210> 10
<211> 132
<212> PRT
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Human IL5
      modified by substitution with tetanus toxoid P30
      epitope

<220>
<221> MUTAGEN
<222> (110)..(130)
<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(129)
<223> Identical to residues 1-129 in SEQ ID NO: 1

<220>
<221> SIMILAR
<222> (131)..(132)
<223> Identical to residues 114-115 in SEQ ID NO: 1

<400> 10
```
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15

Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30

Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35                  40                  45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50                  55                  60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
65                  70                  75                  80

Gly Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val Asn Gln Phe
                85                  90                  95

Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Phe Asn Asn
            100                 105                 110

Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala Ser His
            115                 120                 125

Leu Glu Glu Ser
    130
```

<210> 11
<211> 141
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Human IL5
      modified by substitution with tetanus toxoid P2
      and P30 epitopes

```
<220>
<221> MUTAGEN
<222> (86)..(100)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)


<220>
<221> MUTAGEN
<222> (119)..(139)
<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)


<220>
<221> SIMILAR
<222> (1)..(85)
<223> Identical to residues 1-85 in SEQ ID NO: 1


<220>
<221> SIMILAR
<222> (101)..(118)
<223> Identical to residues 92-109 in SEQ ID NO: 1


<220>
<221> SIMILAR
<222> (140)..(141)
<223> Identical to residues 114-115 in SEQ ID NO: 1


<400> 11
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15

Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30

Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35                  40                  45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50                  55                  60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
65                  70                  75                  80

Gly Gln Lys Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
                85                  90                  95

Ile Thr Glu Leu Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe
                100                 105                 110

Leu Gly Val Met Asn Thr Phe Asn Asn Phe Thr Val Ser Phe Trp Leu
            115                 120                 125

Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Glu Ser
    130                 135                 140


<210> 12
<211> 113
<212> PRT
<213> Mus musculus
```

11

```
<220>
<221> DISULFID
<222> (42)
<223> Interchain disulphide bond to Cys-84 in SEQ ID
      NO:12


<220>
<221> DISULFID
<222> (84)
<223> Interchain disulphide bond to Cys-42 in SEQ ID
      NO:12


<400> 12
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Ala Leu Leu
  1               5              10              15


Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
             20              25              30


Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
         35              40              45


Gly Leu Asp Ile Leu Lys Asp Gln Thr Val Arg Gly Gly Thr Val Met
     50              55              60


Arg Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
 65              70              75              80


Glu Lys Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln Phe Leu Asp
             85              90              95


Tyr Leu Gln Glu Phe Leu Gly Ser Met Asn Thr Ala Ala Ile Ile Glu
            100             105             110


Gly



<210> 13
<211> 124
<212> PRT
<213> Artificial Sequence


<220>
<223> Description of Artificial Sequence:Murine IL5
      modified by substitution with tetanus toxoid P2
      epitope


<220>
<221> MUTAGEN
<222> (85)..(99)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)


<220>
<221> SIMILAR
<222> (1)..(84)
<223> Identical to residues 1-84 in SEQ ID NO: 12


<220>
<221> SIMILAR
```

```
<222>  (100)..(124)
<223>  Identical to residues 89-113 in SEQ ID NO: 12


<400>  13
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Ala Leu Leu
 1               5                  10                  15


Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20                  25                  30


Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
        35                  40                  45


Gly Leu Asp Ile Leu Lys Asp Gln Thr Val Arg Gly Gly Thr Val Met
        50                  55                  60


Arg Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
 65                  70                  75                  80


Glu Lys Lys Cys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile
                85                  90                  95


Thr Glu Leu Arg Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe
            100                 105                 110


Leu Gly Ser Met Asn Thr Ala Ala Ile Ile Glu Gly
            115                 120




<210>  14
<211>  116
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Description of Artificial Sequence:Murine IL5
       modified by substitution with tetanus toxoid P2
       epitope

<220>
<221>  MUTAGEN
<222>  (30)..(44)
<223>  Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221>  SIMILAR
<222>  (1)..(29)
<223>  Identical to residues 1-29 in SEQ ID NO: 12

<220>
<221>  SIMILAR
<222>  (45)..(116)
<223>  Identical to residues 42-113 in SEQ ID NO: 12

<400>  14
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Ala Leu Leu
 1               5                  10                  15


Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Gln Tyr Ile
            20                  25                  30
```

```
Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Cys Ile Gly Glu
         35              40              45

Ile Phe Gln Gly Leu Asp Ile Leu Lys Asp Gln Thr Val Arg Gly Gly
     50              55              60

Thr Val Met Arg Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile
 65              70              75              80

Asp Arg Gln Glu Lys Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln
             85              90              95

Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly Ser Met Asn Thr Ala Ala
             100             105             110

Ile Ile Glu Gly
         115
```

```
<210> 15
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL5
      modified by substitution with tetanus toxoid P2
      epitope

<220>
<221> MUTAGEN
<222> (57)..(71)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221> SIMILAR
<222> (1)..(56)
<223> Identical to residues 1-56 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (72)..(122)
<223> Identical to residues 63-113 in SEQ ID NO: 12

<400> 15
```

```
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Ala Leu Leu
 1               5               10              15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
             20              25              30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
         35              40              45

Gly Leu Asp Ile Leu Lys Asp Gln Gln Tyr Ile Lys Ala Asn Ser Lys
     50              55              60

Phe Ile Gly Ile Thr Glu Leu Val Met Arg Leu Phe Gln Asn Leu Ser
 65              70              75              80
```

```
Leu Ile Lys Lys Tyr Ile Asp Arg Gln Glu Lys Lys Cys Gly Glu Glu
                85                  90                  95

Arg Arg Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
            100                 105                 110

Ser Met Asn Thr Ala Ala Ile Ile Glu Gly
        115                 120     .
```


<210> 16
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL5
      modified by substitution with tetanus toxoid P2
      epitope

<220>
<221> MUTAGEN
<222> (84)..(98)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221> SIMILAR
<222> (1)..(83)
<223> Identical to residues 1-83 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (99)..(122)
<223> Identical to residues 90-113 in SEQ ID NO: 12

<400> 16
```
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Ala Leu Leu
 1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20                  25                  30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
        35                  40                  45

Gly Leu Asp Ile Leu Lys Asp Gln Thr Val Arg Gly Gly Thr Val Met
    50                  55                  60

Arg Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65                  70          .        75                  80

Glu Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr
            85                  90                  95

Glu Leu Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
            100                 105                 110

Ser Met Asn Thr Ala Ala Ile Ile Glu Gly
        115                 120
```

```
<210> 17
<211> 124
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL5
      modified by substitution with tetanus toxoid P2
      epitope

<220>
<221> MUTAGEN
<222> (108)..(122)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221> SIMILAR
<222> (1)..(107)
<223> Identical to residues 1-107 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (123)..(124)
<223> Identical to residues 112-113 in SEQ ID NO: 12

<400> 17
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Ala Leu Leu
 1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20                  25                  30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
        35                  40                  45

Gly Leu Asp Ile Leu Lys Asp Gln Thr Val Arg Gly Gly Thr Val Met
    50                  55                  60

Arg Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65                  70                  75                      80

Glu Lys Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln Phe Leu Asp
                85                  90                      95

Tyr Leu Gln Glu Phe Leu Gly Ser Met Asn Thr Gln Tyr Ile Lys Ala
                100                 105                 110

Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Glu Gly
            115                 120


<210> 18
<211> 130
<212> PRT
<213> Artificial Sequence

<220>
```

```
<223> Description of Artificial Sequence:Murine IL5
      modified by substitution with tetanus toxoid P30
      epitope

<220>
<221> MUTAGEN
<222> (85)..(105)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(84)
<223> Identical to residues 1-84 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (106)..(130)
<223> Identical to residues 89-113 in SEQ ID NO: 12

<400> 18
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Ala Leu Leu
 1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20                  25                  30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
        35                  40                  45

Gly Leu Asp Ile Leu Lys Asp Gln Thr Val Arg Gly Gly Thr Val Met
    50                  55                  60

Arg Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65                  70                  75                  80

Glu Lys Lys Cys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val
                85                  90                  95

Pro Lys Val Ser Ala Ser His Leu Glu Arg Arg Thr Arg Gln Phe Leu
            100                 105                 110

Asp Tyr Leu Gln Glu Phe Leu Gly Ser Met Asn Thr Ala Ala Ile Ile
        115                 120                 125

Glu Gly
    130


<210> 19
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL5
      modified by substitution with tetanus toxoid P30
      epitope

<220>
<221> MUTAGEN
```

17

<222> (30)..(50)
<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(29)
<223> Identical to residues 1-29 in SEQ ID NO: 12

<220>
<221> SIGNAL
<222> (51)..(122)
<223> Identical to residues 42-113 in SEQ ID NO: 12

<400> 19
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Ala Leu Leu
 1   5   10   15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Phe Asn Asn
            20   25   30

Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala Ser His
            35   40   45

Leu Glu Cys Ile Gly Glu Ile Phe Gln Gly Leu Asp Ile Leu Lys Asp
    50   55   60

Gln Thr Val Arg Gly Gly Thr Val Met Arg Leu Phe Gln Asn Leu Ser
65   70   75   80

Leu Ile Lys Lys Tyr Ile Asp Arg Gln Glu Lys Lys Cys Gly Glu Glu
                85   90   95

Arg Arg Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
            100   105   110

Ser Met Asn Thr Ala Ala Ile Ile Glu Gly
            115   120

<210> 20
<211> 128
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL5
      modified by substitution with tetanus toxoid P30
      epitope

<220>
<221> MUTAGEN
<222> (57)..(77)
<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(56)
<223> Identical to residues 1-56 in SEQ ID NO: 12

<220>

```
<221> SIMILAR
<222> (78)..(128)
<223> Identical to residues 63-113 in SEQ ID NO: 12


<400> 20
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Ala Leu Leu
  1               5               10               15


Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
             20               25               30


Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
         35               40               45


Gly Leu Asp Ile Leu Lys Asp Gln Phe Asn Asn Phe Thr Val Ser Phe
     50               55               60                  .


Trp Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Val Met Arg
 65               70               75               80


Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln Glu
                 85               90               95


Lys Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln Phe Leu Asp Tyr
             100              105              110


Leu Gln Glu Phe Leu Gly Ser Met Asn Thr Ala Ala Ile Ile Glu Gly
         115              120              125
```

```
<210> 21
<211> 130
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL5
      modified by substitution with tetanus toxoid P30
      epitope

<220>
<221> MUTAGEN
<222> (108)..(128)
<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(107)
<223> Identical to residues 1-107 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (129)..(130)
<223> Identical to residues 112-113 in SEQ ID NO: 12

<400> 21
```

```
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Ala Leu Leu
 1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20                  25                  30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
        35                  40                  45

Gly Leu Asp Ile Leu Lys Asp Gln Thr Val Arg Gly Gly Thr Val Met
    50                  55                  60

Arg Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65                  70                  75                  80

Glu Lys Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln Phe Leu Asp
            85                  90                  95

Tyr Leu Gln Glu Phe Leu Gly Ser Met Asn Thr Phe Asn Asn Phe Thr
            100                 105                 110

Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu
        115                 120                 125


Glu Gly
    130
```

<210> 22
<211> 139
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Murine IL5
      modified by substitution with tetanus toxoid P2
      and P30 epitopes

<220>
<221> MUTAGEN
<222> (84)..(98)
<223> Tetanus toxoid P2 epitope (SEQ ID NO: 23)

<220>
<221> MUTAGEN
<222> (117)..(137)
<223> Tetanus toxoid P30 epitope (SEQ ID NO: 24)

<220>
<221> SIMILAR
<222> (1)..(83)
<223> Identical to residues 1-83 in SEQ ID NO: 12

<220>
<221> SIMILAR
<222> (99)..(116)
<223> Identical to residues 90-109 in SEQ ID NO: 12

<220>

```
<221> SIMILAR
<222> (138)..(139)
<223> Identical to residues 112-113 in SEQ ID NO: 12


<400> 22
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Ala Leu Leu
 1               5                  10                  15


Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20                  25                  30


Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
        35                  40                  45


Gly Leu Asp Ile Leu Lys Asp Gln Thr Val Arg Gly Gly Thr Val Met
    50                  55                  60


Arg Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65                  70                  75                  80


Glu Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr
                85                  90                  95


Glu Leu Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
            100                 105                 110


Ser Met Asn Thr Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val
            115                 120                 125


Pro Lys Val Ser Ala Ser His Leu Glu Glu Gly
        130                 135



<210> 23
<211> 15
<212> PRT
<213> Clostridium tetani

<400> 23
Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu
 1               5                  10                  15



<210> 24
<211> 21
<212> PRT
<213> Clostridium tetani

<400> 24
Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser
 1               5                  10                  15


Ala Ser His Leu Glu
            20



<210> 25
<211> 45
```

```
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(45)

<220>
<223> Description of Artificial sequence: DNA encoding His tag

<400> 25
atg aaa cac caa cac caa cat caa cat caa cat caa cat caa caa      45
Met Lys His Gln His Gln His Gln His Gln His Gln His Gln Gln
 1               5               10              15


<210> 26
<211> 15
<212> PRT
<213> Artificial Sequence

<400> 26
Met Lys His Gln His Gln His Gln His Gln His Gln His Gln Gln
 1               5               10              15



<210> 27
<211> 381
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(381)

<220>
<221> mutation
<222> (262)..(306)
<223> Tetanus toxoid P2 epitope

<220>
<221> misc_feature
<222> (1)..(261)
<223> DNA encoding amino acids 1-87 of human IL5

<220>
<221> misc_feature
<222> (307)..(378)
<223> DNA encoding amino acids 92-115 of human IL5

<400> 27
atc ccc aca gaa att ccc aca agt gca ttg gtg aaa gag acc ttg gca      48
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5               10              15
```

22

```
ctg ctt tct act cat cga act ctg ctg ata gcc aat gag act ctc cgg    96
Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30

att cct gtt cct gta cat aaa aat cac caa ctg tgc act gaa gaa atc   144
Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35                  40                  45

ttt cag gga ata ggc aca ctc gag agt caa act gtg caa ggg ggt act   192
Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50                  55                  60

gtg gaa aga cta ttc aaa aac ttg tcc tta ata aag aaa tac atc gat   240
Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
65                  70                  75                  80

ggc caa aaa aaa aag tgt gga cag tac atc aag gcc aac tcc aag ttc   288
Gly Gln Lys Lys Lys Cys Gly Gln Tyr Ile Lys Ala Asn Ser Lys Phe
                85                  90                  95

atc ggc atc acc gag ctg aga gta aac caa ttc cta gac tat ctg cag   336
Ile Gly Ile Thr Glu Leu Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln
            100                 105                 110

gag ttt ctt ggt gta atg aac acc gag tgg ata ata gaa agt tga       381
Glu Phe Leu Gly Val Met Asn Thr Glu Trp Ile Ile Glu Ser
        115                 120                 125
```

<210> 28
<211> 126
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<400> 28
```
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15

Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30

Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35                  40                  45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50                  55                  60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
65                  70                  75                  80

Gly Gln Lys Lys Lys Cys Gly Gln Tyr Ile Lys Ala Asn Ser Lys Phe
                85                  90                  95

Ile Gly Ile Thr Glu Leu Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln
            100                 105                 110

Glu Phe Leu Gly Val Met Asn Thr Glu Trp Ile Ile Glu Ser
        115                 120                 125
```

23

```
<210> 29
<211> 375
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(375)

<220>
<221> mutation
<222> (94)..(156)
<223> Tetanus toxoid P30 epitope

<220>
<221> misc_feature
<222> (1)..(93)
<223> DNA encoding amino acids 1-31 of human IL5

<220>
<221> misc_feature
<222> (157)..(372)
<223> DNA encoding amino acids 44-115 of human IL5

<400> 29
atc ccc aca gaa att ccc aca agt gca ttg gtg aaa gag acc ttg gca    48
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15

ctg ctt tct act cat cga act ctg ctg ata gcc aat gag act ctc ttc    96
Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Phe
            20                  25                  30

aac aac ttc acc gtg agc ttc tgg ctg cgc gtg cct aag gtg agc gcc   144
Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala
            35                  40                  45

agc cac ctg gag tgc act gaa gaa atc ttt cag gga ata ggc aca ctc   192
Ser His Leu Glu Cys Thr Glu Glu Ile Phe Gln Gly Ile Gly Thr Leu
        50                  55                  60

gag agt caa act gtg caa ggg ggt act gtg gaa aga cta ttc aaa aac   240
Glu Ser Gln Thr Val Gln Gly Gly Thr Val Glu Arg Leu Phe Lys Asn
65                  70                  75                  80

ttg tcc tta ata aag aaa tac atc gat ggc caa aaa aaa aag tgt gga   288
Leu Ser Leu Ile Lys Lys Tyr Ile Asp Gly Gln Lys Lys Lys Cys Gly
                85                  90                  95

gaa gaa aga cgg aga gta aac caa ttc cta gac tat ctg cag gag ttt   336
Glu Glu Arg Arg Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe
            100                 105                 110
```

24

```
ctt ggt gta atg aac acc gag tgg ata ata gaa agt tga          375
Leu Gly Val Met Asn Thr Glu Trp Ile Ile Glu Ser
        115                 120
```

```
<210> 30
<211> 124
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<400> 30
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                   10                  15


Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Phe
            20                  25                  30


Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala
        35                  40                  45


Ser His Leu Glu Cys Thr Glu Glu Ile Phe Gln Gly Ile Gly Thr Leu
    50                  55                  60


Glu Ser Gln Thr Val Gln Gly Gly Thr Val Glu Arg Leu Phe Lys Asn
65                  70                  75                  80


Leu Ser Leu Ile Lys Lys Tyr Ile Asp Gly Gln Lys Lys Lys Cys Gly
                85                  90                  95


Glu Glu Arg Arg Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe
            100                 105                 110


Leu Gly Val Met Asn Thr Glu Trp Ile Ile Glu Ser
        115                 120
```

```
<210> 31
<211> 393
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(393)

<220>
<221> mutation
<222> (175)..(237)
<223> Tetanus toxoid P30 epitope

<220>
```

```
<221> misc_feature
<222> (1)..(174)
<223> DNA encoding amino acids 1-58 of human IL5

<220>
<221> misc_feature
<222> (238)..(390)
<223> DNA encoding amino acids 65-115 of human IL5


<400> 31
atc ccc aca gaa att ccc aca agt gca ttg gtg aaa gag acc ttg gca      48
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15

ctg ctt tct act cat cga act ctg ctg ata gcc aat gag act ctc cgg      96
Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30

att cct gtt cct gta cat aaa aat cac caa ctg tgc act gaa gaa atc     144
Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35                  40                  45

ttt cag gga ata ggc aca ctc gag agt caa ttc aac aac ttc acc gtg     192
Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Phe Asn Asn Phe Thr Val
    50                  55                  60

agc ttc tgg ctg cgc gtg cct aag gtg agc gcc agc cac ctg gag gtg     240
Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Val
65                  70                  75                  80

gaa aga cta ttc aaa aac ttg tcc tta ata aag aaa tac atc gat ggc     288
Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Gly
                85                  90                  95

caa aaa aaa aag tgt gga gaa gaa aga cgg aga gta aac caa ttc cta     336
Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val Asn Gln Phe Leu
               100                 105                 110

gac tat ctg cag gag ttt ctt ggt gta atg aac acc gag tgg ata ata     384
Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Glu Trp Ile Ile
           115                 120                 125

gaa agt tga                                                         393
Glu Ser
   130


<210> 32
<211> 130
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope


<400> 32
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15

Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30
```

```
Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35              40              45
```

```
Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Phe Asn Asn Phe Thr Val
        50              55              60
```

```
Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Val
65                  70              75                      80
```

```
Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Gly
                85              90                  95
```

```
Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val Asn Gln Phe Leu
            100             105             110
```

```
Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Glu Trp Ile Ile
        115             120             125
```

```
Glu Ser
    130
```

<210> 33
<211> 375
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(375)

<220>
<221> mutation
<222> (175)..(219)
<223> Tetanus toxoid P2 epitope

<220>
<221> misc_feature
<222> (1)..(174)
<223> DNA encoding amino acids 1-58 of human IL5

<220>
<221> misc_feature
<222> (220)..(372)
<223> DNA encoding amino acids 65-115 of human IL5

<400> 33
```
atc ccc aca gaa att ccc aca agt gca ttg gtg aaa gag acc ttg gca    48
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15
```

```
ctg ctt tct act cat cga act ctg ctg ata gcc aat gag act ctc cgg    96
Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20              25                  30
```

```
att cct gtt cct gta cat aaa aat cac caa ctg tgc act gaa gaa atc     144
Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35                  40                  45

ttt cag gga ata ggc aca ctc gag agt caa cag tac atc aag gcc aac     192
Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Gln Tyr Ile Lys Ala Asn
    50                  55                  60

tcc aag ttc atc ggc atc acc gag ctg gtg gaa aga cta ttc aaa aac     240
Ser Lys Phe Ile Gly Ile Thr Glu Leu Val Glu Arg Leu Phe Lys Asn
65                  70                  75                  80

ttg tcc tta ata aag aaa tac atc gat ggc caa aaa aaa aag tgt gga     288
Leu Ser Leu Ile Lys Lys Tyr Ile Asp Gly Gln Lys Lys Lys Cys Gly
                85                  90                  95

gaa gaa aga cgg aga gta aac caa ttc cta gac tat ctg cag gag ttt     336
Glu Glu Arg Arg Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe
            100                 105                 110

ctt ggt gta atg aac acc gag tgg ata ata gaa agt tga               375
Leu Gly Val Met Asn Thr Glu Trp Ile Ile Glu Ser
        115                 120
```

```
<210> 34
<211> 124
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Human Il-5

     modified by substitution with tetanus toxoid
     epitope

<400> 34
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15

Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30

Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35                  40                  45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Gln Tyr Ile Lys Ala Asn
    50                  55                  60

Ser Lys Phe Ile Gly Ile Thr Glu Leu Val Glu Arg Leu Phe Lys Asn
65                  70                  75                  80

Leu Ser Leu Ile Lys Lys Tyr Ile Asp Gly Gln Lys Lys Lys Cys Gly
                85                  90                  95

Glu Glu Arg Arg Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe
            100                 105                 110

Leu Gly Val Met Asn Thr Glu Trp Ile Ile Glu Ser
        115                 120
```

<210> 35
<211> 357
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(357)

<220>
<221> mutation
<222> (94)..(138)
<223> Tetanus toxoid P2 epitope

<220>
<221> misc_feature
<222> (1)..(93)
<223> DNA encoding amino acids 1-31 of human IL5

<220>
<221> misc_feature
<222> (139)..(354)
<223> DNA encoding amino acids 44-115 of human IL5

<400> 35
atc ccc aca gaa att ccc aca agt gca ttg gtg aaa gag acc ttg gca   48
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
  1   5   10   15

ctg ctt tct act cat cga act ctg ctg ata gcc aat gag act ctc cag   96
Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Gln
                 20   25   30

tac atc aag gcc aac tcc aag ttc atc ggc atc acc gag ctg tgc act   144
Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Cys Thr
             35   40   45

gaa gaa atc ttt cag gga ata ggc aca ctc gag agt caa act gtg caa   192
Glu Glu Ile Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln
         50   55   60

ggg ggt act gtg gaa aga cta ttc aaa aac ttg tcc tta ata aag aaa   240
Gly Gly Thr Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys
 65   70   75   80

tac atc gat ggc caa aaa aaa aag tgt gga gaa gaa aga cgg aga gta   288
Tyr Ile Asp Gly Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val
                 85   90   95

aac caa ttc cta gac tat ctg cag gag ttt ctt ggt gta atg aac acc   336
Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr
                100   105   110

```
gag tgg ata ata gaa agt tga                                  357
Glu Trp Ile Ile Glu Ser
        115
```

<210> 36
<211> 118
<212> PRT
<213> Artificial Sequence

<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<400> 36
```
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15

Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Gln
            20                  25                  30

Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Cys Thr
        35        .              40                  45

Glu Glu Ile Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln
    50                  55                  60

Gly Gly Thr Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys
65                  70                  75                  80

Tyr Ile Asp Gly Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val
                85                  90                  95

Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr
            100                 105                 110

Glu Trp Ile Ile Glu Ser
        115
```

<210> 37
<211> 375
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(375)

<220>
<221> mutation
<222> (256)..(300)
<223> Tetanus toxoid P2 epitope

<220>

```
<221> misc_feature
<222> (1)..(255)
<223> DNA encoding amino acids 1-85 of human IL5


<220>
<221> misc_feature
<222> (301)..(372)
<223> DNA encoding amino acids 92-115 of human IL5


<400> 37
atc ccc aca gaa att ccc aca agt gca ttg gtg aaa gag acc ttg gca        48
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15

ctg ctt tct act cat cga act ctg ctg ata gcc aat gag act ctc cgg        96
Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
                20                  25                  30

att cct gtt cct gta cat aaa aat cac caa ctg tgc act gaa gaa atc       144
Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
            35                  40                  45

ttt cag gga ata ggc aca ctc gag agt caa act gtg caa ggg ggt act       192
Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
        50                  55                  60

gtg gaa aga cta ttc aaa aac ttg tcc tta ata aag aaa tac atc gat       240
Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
 65                 70                  75                  80

ggc caa aaa aaa aag cag tac atc aag gcc aac tcc aag ttc atc ggc       288
Gly Gln Lys Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
                85                  90                  95

atc acc gag ctg aga gta aac caa ttc cta gac tat ctg cag gag ttt       336
Ile Thr Glu Leu Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe
                100                 105                 110

ctt ggt gta atg aac acc gag tgg ata ata gaa agt tga                   375
Leu Gly Val Met Asn Thr Glu Trp Ile Ile Glu Ser
        115                 120


<210> 38
<211> 124
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope


<400> 38
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15


Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
                20                  25                  30


Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
            35                  40                  45
```

```
Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
        50              55                  60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
 65              70                  75                  80

Gly Gln Lys Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
                85                  90                  95

Ile Thr Glu Leu Arg Val Asn Gln Phe Leu Asp Tyr Leu Gln Glu Phe
            100                 105                 110

Leu Gly Val Met Asn Thr Glu Trp Ile Ile Glu Ser
            115                 120
```

```
<210> 39
<211> 399
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(399)

<220>
<221> mutation
<222> (262)..(324)
<223> Tetanus toxoid P30 epitope

<220>
<221> misc_feature
<222> (1)..(261)
<223> DNA encoding amino acids 1-87 of human IL5

<220>
<221> misc_feature
<222> (325)..(396)
<223> DNA encoding amino acids 92-115 of human IL5

<400> 39
atc ccc aca gaa att ccc aca agt gca ttg gtg aaa gag acc ttg gca      48
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15

ctg ctt tct act cat cga act ctg ctg ata gcc aat gag act ctc cgg      96
Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30

att cct gtt cct gta cat aaa aat cac caa ctg tgc act gaa gaa atc     144
Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
            35                  40                  45
```

32

```
ttt cag gga ata ggc aca ctc gag agt caa act gtg caa ggg ggt act    192
Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50                  55                  60

gtg gaa aga cta ttc aaa aac ttg tcc tta ata aag aaa tac atc gat    240
Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
65                  70                  75                  80

ggc caa aaa aaa aag tgt gga ttc aac aac ttc acc gtg agc ttc tgg    288
Gly Gln Lys Lys Lys Cys Gly Phe Asn Asn Phe Thr Val Ser Phe Trp
                85                  90                  95

ctg cgc gtg cct aag gtg agc gcc agc cac ctg gag aga gta aac caa    336
Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Arg Val Asn Gln
            100                 105                 110

ttc cta gac tat ctg cag gag ttt ctt ggt gta atg aac acc gag tgg    384
Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Glu Trp
            115                 120                 125

ata ata gaa agt tga                                               399
Ile Ile Glu Ser
    130
```

```
<210> 40
<211> 132
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<400> 40
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
  1               5                  10                  15

Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30

Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35                  40                  45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50                  55                  60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
65                  70                  75                  80

Gly Gln Lys Lys Lys Cys Gly Phe Asn Asn Phe Thr Val Ser Phe Trp
                85                  90                  95

Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Arg Val Asn Gln
            100                 105                 110

Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Glu Trp
            115                 120                 125

Ile Ile Glu Ser
    130
```

```
<210> 41
<211> 393
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(393)

<220>
<221> mutation
<222> (256)..(318)
<223> Tetanus toxoid P30 epitope

<220>
<221> misc_feature
<222> (1)..(255)
<223> DNA encoding amino acids 1-85 of human IL5

<220>
<221> misc_feature
<222> (319)..(390)
<223> DNA encoding amino acids 92-115 of human IL5

<400> 41
atc ccc aca gaa att ccc aca agt gca ttg gtg aaa gag acc ttg gca      48
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
  1               5                  10                  15

ctg ctt tct act cat cga act ctg ctg ata gcc aat gag act ctc cgg      96
Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
             20                  25                  30

att cct gtt cct gta cat aaa aat cac caa ctg tgc act gaa gaa atc     144
Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
         35                  40                  45

ttt cag gga ata ggc aca ctc gag agt caa act gtg caa ggg ggt act     192
Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
     50                  55                  60

gtg gaa aga cta ttc aaa aac ttg tcc tta ata aag aaa tac atc gat     240
Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
65                  70                  75                  80

ggc caa aaa aaa aag ttc aac aac ttc acc gtg agc ttc tgg ctg cgc     288
Gly Gln Lys Lys Lys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg
                 85                  90                  95

gtg cct aag gtg agc gcc agc cac ctg gag aga gta aac caa ttc cta     336
Val Pro Lys Val Ser Ala Ser His Leu Glu Arg Val Asn Gln Phe Leu
                100                 105                 110
```

34

```
gac tat ctg cag gag ttt ctt ggt gta atg aac acc gag tgg ata ata    384
Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Glu Trp Ile Ile
        115                 120                 125


gaa agt tga                                                         393
Glu Ser
    130



<210> 42
<211> 130
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitope

<400> 42
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
  1               5                 10                  15


Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30


Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35                  40                  45


Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50                  55                  60


Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
 65                  70                  75                  80


Gly Gln Lys Lys Lys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg
                85                  90                  95


Val Pro Lys Val Ser Ala Ser His Leu Glu Arg Val Asn Gln Phe Leu
            100                 105                 110


Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Glu Trp Ile Ile
        115                 120                 125


Glu Ser
    130



<210> 43
<211> 444
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitopes

<220>
<221> CDS
<222> (1)..(444)
```

```
<220>
<221> mutation
<222> (262)..(306)
<223> Tetanus toxoid P2 epitope

<220>
<221> mutation
<222> (307)..(369)
<223> Tetanus toxoid P30 epitope

<220>
<221> misc_feature
<222> (1)..(261)
<223> DNA encoding amino acids 1-87 of human IL5

<220>
<221> misc_feature
<222> (370)..(441)
<223> DNA encoding amino acids 92-115 of human IL5

<400> 43
atc ccc aca gaa att ccc aca agt gca ttg gtg aaa gag acc ttg gca    48
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                  10                  15

ctg ctt tct act cat cga act ctg ctg ata gcc aat gag act ctc cgg    96
Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30

att cct gtt cct gta cat aaa aat cac caa ctg tgc act gaa gaa atc    144
Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35                  40                  45

ttt cag gga ata ggc aca ctc gag agt caa act gtg caa ggg ggt act    192
Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50                  55                  60

gtg gaa aga cta ttc aaa aac ttg tcc tta ata aag aaa tac atc gat    240
Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
 65                  70                  75                  80

ggc caa aaa aaa aag tgt gga cag tac atc aag gcc aac tcc aag ttc    288
Gly Gln Lys Lys Lys Cys Gly Gln Tyr Ile Lys Ala Asn Ser Lys Phe
                85                  90                  95

atc ggc atc acc gag ctg ttc aac aac ttc acc gtg agc ttc tgg ctg    336
Ile Gly Ile Thr Glu Leu Phe Asn Asn Phe Thr Val Ser Phe Trp Leu
            100                 105                 110

cgc gtg cct aag gtg agc gcc agc cac ctg gag aga gta aac caa ttc    384
Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Arg Val Asn Gln Phe
        115                 120                 125

cta gac tat ctg cag gag ttt ctt ggt gta atg aac acc gag tgg ata    432
Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Glu Trp Ile
        130                 135                 140

ata gaa agt tga                                                    444
Ile Glu Ser
145
```

36

```
<210> 44
<211> 147
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Human Il-5
      modified by substitution with tetanus toxoid
      epitopes

<400> 44
Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1               5                   10                  15

Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20                  25                  30

Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
            35                  40                  45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50                  55                  60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
 65                 70                  75                  80

Gly Gln Lys Lys Lys Cys Gly Gln Tyr Ile Lys Ala Asn Ser Lys Phe
                85                  90                  95

Ile Gly Ile Thr Glu Leu Phe Asn Asn Phe Thr Val Ser Phe Trp Leu
            100                 105                 110

Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Arg Val Asn Gln Phe
            115                 120                 125

Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Glu Trp Ile
    130                 135                 140

Ile Glu Ser
145


<210> 45
<211> 375
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(375)

<220>
<221> mutation
<222> (256)..(300)
<223> Tetanus toxoid P2 epitope
```

```
<220>
<221> misc_feature
<222> (1)..(255)
<223> DNA encoding amino acids 1-85 in murine IL5

<220>
<221> misc_feature
<222> (301)..(375)
<223> DNA encoding amino acids 90-113 of murine IL5

<400> 45
atg gag att ccc atg agc aca gtg gtg aaa gag acc ttg aca cag ctg    48
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
 1               5                  10                  15

tcc gct cac cga gct ctg ttg aca agc aat gag acg atg agg ctt cct    96
Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
             20                  25                  30

gtc cct act cat aaa aat cac cag cta tgc att gga gag atc ttt cag   144
Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
         35                  40                  45

ggg cta gac ata ctg aag aat caa act gtc cgt ggg ggt acc gtg gaa   192
Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
     50                  55                  60

atg cta ttc caa aac ctg tca tta ata aag aaa tac atc gat aga caa   240
Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
 65                  70                  75                  80

aaa gag aag tgt ggc cag tac atc aaa gct aac tcc aaa ttc atc ggt   288
Lys Glu Lys Cys Gly Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
                 85                  90                  95

atc acc gag ctg agg acg agg cag ttc ctg gat tat ctg cag gag ttc   336
Ile Thr Glu Leu Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe
             100                 105                 110

ctt ggt gtg atg agt aca gag tgg gca atg gaa ggc taa               375
Leu Gly Val Met Ser Thr Glu Trp Ala Met Glu Gly
         115                 120


<210> 46
<211> 124
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitope

<400> 46
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
 1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
             20                  25                  30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
         35                  40                  45
```

```
Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
    50                  55                  60

Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65                  70                  75                  80

Lys Glu Lys Cys Gly Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
                85                  90                  95

Ile Thr Glu Leu Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe
            100                 105                 110

Leu Gly Val Met Ser Thr Glu Trp Ala Met Glu Gly
        115                 120
```

```
<210> 47
<211> 369
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(369)

<220>
<221> mutation
<222> (88)..(150)
<223> Tetanus toxoid P30 epitope

<220>
<221> misc_feature
<222> (1)..(87)
<223> DNA encoding amino acids 1-29 of murine IL5

<220>
<221> misc_feature
<222> (151)..(366)
<223> DNA encoding amino acids 42-113 of murine IL5

<400> 47
atg gag att ccc atg agc aca gtg gtg aaa gag acc ttg aca cag ctg    48
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
  1               5                  10                  15

tcc gct cac cga gct ctg ttg aca agc aat gag acg atg ttc aac aac    96
Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Phe Asn Asn
                20                  25                  30

ttc acc gtg agc ttc tgg ctg cgc gtg ccc aag gtg agc gcc agc cac   144
Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala Ser His
                35                  40                  45
```

```
ctg gag tgc att gga gag atc ttt cag ggg cta gac ata ctg aag aat      192
Leu Glu Cys Ile Gly Glu Ile Phe Gln Gly Leu Asp Ile Leu Lys Asn
     50              55              60

caa act gtc cgt ggg ggt acc gtg gaa atg cta ttc caa aac ctg tca      240
Gln Thr Val Arg Gly Gly Thr Val Glu Met Leu Phe Gln Asn Leu Ser
 65              70              75              80

tta ata aag aaa tac atc gat aga caa aaa gag aag tgt ggc gag gag      288
Leu Ile Lys Lys Tyr Ile Asp Arg Gln Lys Glu Lys Cys Gly Glu Glu
                 85              90              95

aga cgg agg acg agg cag ttc ctg gat tat ctg cag gag ttc ctt ggt      336
Arg Arg Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
                100             105             110

gtg atg agt aca gag tgg gca atg gaa ggc taa                          369
Val Met Ser Thr Glu Trp Ala Met Glu Gly
            115             120
```

<210> 48
<211> 122
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Murine Il-5
     modified by substitution with tetanus toxoid
     epitope

<400> 48
```
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
 1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Phe Asn Asn
            20              25              30

Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala Ser His
            35              40              45

Leu Glu Cys Ile Gly Glu Ile Phe Gln Gly Leu Asp Ile Leu Lys Asn
     50              55              60

Gln Thr Val Arg Gly Gly Thr Val Glu Met Leu Phe Gln Asn Leu Ser
 65              70              75              80

Leu Ile Lys Lys Tyr Ile Asp Arg Gln Lys Glu Lys Cys Gly Glu Glu
                 85              90              95

Arg Arg Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
                100             105             110

Val Met Ser Thr Glu Trp Ala Met Glu Gly
            115             120
```

<210> 49
<211> 387
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(387)

<220>
<221> mutation
<222> (169)..(231)
<223> Tetanus toxoid P2 epitope

<220>
<221> misc_feature
<222> (1)..(168)
<223> DNA encoding amino acids 1-56 of murine IL5

<220>
<221> misc_feature
<222> (232)..(384)
<223> DNA encoding amino acids 63-113 of murine IL5

<400> 49
atg gag att ccc atg agc aca gtg gtg aaa gag acc ttg aca cag ctg   48
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
 1   5   10   15

tcc gct cac cga gct ctg ttg aca agc aat gag acg atg agg ctt cct   96
Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20   25   30

gtc cct act cat aaa aat cac cag cta tgc att gga gag atc ttt cag   144
Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
        35   40   45

ggg cta gac ata ctg aag aat caa ttc aac aac ttc acc gtg agc ttc   192
Gly Leu Asp Ile Leu Lys Asn Gln Phe Asn Asn Phe Thr Val Ser Phe
    50   55   60

tgg ctg cgc gtg ccc aag gtg agc gcc agc cac ctg gag gtg gaa atg   240
Trp Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Val Glu Met
65   70   75   80

cta ttc caa aac ctg tca tta ata aag aaa tac atc gat aga caa aaa   288
Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln Lys
                85   90   95

gag aag tgt ggc gag gag aga cgg agg acg agg cag ttc ctg gat tat   336
Glu Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln Phe Leu Asp Tyr
            100   105   110

ctg cag gag ttc ctt ggt gtg atg agt aca gag tgg gca atg gaa ggc   384
Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp Ala Met Glu Gly
        115   120   125

taa   387

```
<210> 50
<211> 128
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitope

<400> 50
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
 1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20                  25                  30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
            35                  40                  45

Gly Leu Asp Ile Leu Lys Asn Gln Phe Asn Asn Phe Thr Val Ser Phe
        50                  55                  60

Trp Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Val Glu Met
65                  70                  75                  80

Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln Lys
                85                  90                  95

Glu Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln Phe Leu Asp Tyr
            100                 105                 110

Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp Ala Met Glu Gly
            115                 120                 125


<210> 51
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(351)

<220>
<221> mutation
<222> (88)..(132)
<223> Tetanus toxoid P2 epitope

<220>
<221> misc_feature
<222> (1)..(87)
<223> DNA encoding amino acids 1-29 of murine IL5

<220>
```

```
<221> misc_feature
<222> (133)..(348)
<223> DNA encoding amino acids 42-113 of murine IL5


<400> 51
atg gag att ccc atg agc aca gtg gtg aaa gag acc ttg aca cag ctg    48
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
 1               5                  10        ·         15


tcc gct cac cga gct ctg ttg aca agc aat gag acg atg cag tac atc    96
Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Gln Tyr Ile
             20                  25                  30


aaa gct aac tcc aaa ttc atc ggt atc acc gag ctg tgc att gga gag   144
Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Cys Ile Gly Glu
         35                  40                  45


atc ttt cag ggg cta gac ata ctg aag aat caa act gtc cgt ggg ggt   192
Ile Phe Gln Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly
     50                  55                  60


acc gtg gaa atg cta ttc caa aac ctg tca tta ata aag aaa tac atc   240
Thr Val Glu Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile
 65                  70                  75                  80


gat aga caa aaa gag aag tgt ggc gag gag aga cgg agg acg agg cag   288
Asp Arg Gln Lys Glu Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln
                 85                  90                  95


ttc ctg gat tat ctg cag gag ttc ctt ggt gtg atg agt aca gag tgg   336
Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp
             100                 105                 110


gca atg gaa ggc taa                                                351
Ala Met Glu Gly
             115



<210> 52
<211> 116
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitope


<400> 52
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
 1               5                  10                  15


Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Gln Tyr Ile
             20                  25                  30


Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Cys Ile Gly Glu
         35                  40                  45


Ile Phe Gln Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly
     50                  55                  60


Thr Val Glu Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile
 65                  70                  75                  80
```

```
Asp Arg Gln Lys Glu Lys Cys Gly Glu Glu Arg Arg Arg Thr Arg Gln
                85                  90                  95

Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp
            100                 105                 110

Ala Met Glu Gly
        115
```

```
<210> 53
<211> 369
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(369)

<220>
<221> mutation
<222> (250)..(294)
<223> Tetanus toxoid P2 epitope

<220>
<221> misc_feature
<222> (1)..(249)
<223> DNA encoding amino acids 1-83 of murine IL5

<220>
<221> misc_feature
<222> (295)..(366)
<223> DNA encoding amino acids 90-113 of murine IL5

<400> 53
atg gag att ccc atg agc aca gtg gtg aaa gag acc ttg aca cag ctg    48
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
  1               5                  10                  15

tcc gct cac cga gct ctg ttg aca agc aat gag acg atg agg ctt cct    96
Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
                20                  25                  30

gtc cct act cat aaa aat cac cag cta tgc att gga gag atc ttt cag   144
Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
            35                  40                  45

ggg cta gac ata ctg aag aat caa act gtc cgt ggg ggt acc gtg gaa   192
Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
        50                  55                  60

atg cta ttc caa aac ctg tca tta ata aag aaa tac atc gat aga caa   240
Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
  65                  70                  75                  80
```

44

```
aaa gag aag cag tac atc aag gcc aac tcc aag ttc atc ggc atc acc   288
Lys Glu Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr
                85                  90                  95

gag ctg agg acg agg cag ttc ctg gat tat ctg cag gag ttc ctt ggt   336
Glu Leu Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
            100                 105                 110

gtg atg agt aca gag tgg gca atg gaa ggc taa                       369
Val Met Ser Thr Glu Trp Ala Met Glu Gly
            115                 120
```

<210> 54
<211> 122
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid

 epitope

<400> 54
```
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
  1             5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
                20                  25                  30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
                35                  40                  45

Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
            50                  55                  60

Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
 65                  70                  75                  80

Lys Glu Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr
                85                  90                  95

Glu Leu Arg Thr Arg Gln Phe Leu Asp Tyr Leu Gln Glu Phe Leu Gly
            100                 105                 110

Val Met Ser Thr Glu Trp Ala Met Glu Gly
            115                 120
```

<210> 55
<211> 393
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>

```
<221> CDS
<222> (1)..(393)

<220>
<221> mutation
<222> (256)..(318)
<223> Tetanus toxoid P30 epitope

<220>
<221> misc_feature
<222> (1)..(255)
<223> DNA encoding amino acids 1-85 of murine IL5

<220>
<221> misc_feature
<222> (319)..(390)
<223> DNA encoding amino acids 90-113 of murine IL5

<400> 55
atg gag att ccc atg agc aca gtg gtg aaa gag acc ttg aca cag ctg      48
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
  1               5                  10                  15

tcc gct cac cga gct ctg ttg aca agc aat gag acg atg agg ctt cct      96
Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
                20                  25                  30

gtc cct act cat aaa aat cac cag cta tgc att gga gag atc ttt cag     144
Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
            35                  40                  45

ggg cta gac ata ctg aag aat caa act gtc cgt ggg ggt acc gtg gaa     192
Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
        50                  55                  60

atg cta ttc caa aac ctg tca tta ata aag aaa tac atc gat aga caa     240
Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
 65                  70                  75                  80

aaa gag aag tgt ggc ttc aac aac ttc acc gtg agc ttc tgg ctg cgc     288
Lys Glu Lys Cys Gly Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg
                    85                  90                  95

gtg ccc aag gtg agc gcc agc cac ctg gag agg acg agg cag ttc ctg     336
Val Pro Lys Val Ser Ala Ser His Leu Glu Arg Thr Arg Gln Phe Leu
                100                 105                 110

gat tat ctg cag gag ttc ctt ggt gtg atg agt aca gag tgg gca atg     384
Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp Ala Met
            115                 120                 125

gaa ggc taa                                                         393
Glu Gly
        130


<210> 56
<211> 130
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Murine Il-5
```

46

modified by substitution with tetanus toxoid
epitope

<400> 56

```
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
 1               5                  10                  15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20                  25                  30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
         35                  40                  45

Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
      50                  55                  60

Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
 65                  70                  75                  80

Lys Glu Lys Cys Gly Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg
               85                  90                  95

Val Pro Lys Val Ser Ala Ser His Leu Glu Arg Thr Arg Gln Phe Leu
            100                 105                 110

Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp Ala Met
            115                 120                 125

Glu Gly
    130
```

<210> 57
<211> 387
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitope

<220>
<221> CDS
<222> (1)..(387)   ·

<220>
<221> mutation
<222> (250)..(312)
<223> Tetanus toxoid P30 epitope

<220>
<221> misc_feature
<222> (1)..(249)
<223> DNA encoding amino acids 1-83 of murine IL5

<220>
<221> misc_feature
<222> (313)..(384)
<223> DNA encoding amino acids 90-113 of murine IL5   ·

47

```
<400> 57
atg gag att ccc atg agc aca gtg gtg aaa gag acc ttg aca cag ctg    48
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
1               5               10                  15


tcc gct cac cga gct ctg ttg aca agc aat gag acg atg agg ctt cct    96
Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20              25              30


gtc cct act cat aaa aat cac cag cta tgc att gga gag atc ttt cag    144
Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
        35              40              45


ggg cta gac ata ctg aag aat caa act gtc cgt ggg ggt acc gtg gaa    192
Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
    50              55              60


atg cta ttc caa aac ctg tca tta ata aag aaa tac atc gat aga caa    240
Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65              70              75              80


aaa gag aag ttc aac aac ttc acc gtg agc ttc tgg ctg cgc gtg ccc    288
Lys Glu Lys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro
                85              90              95


aag gtg agc gcc agc cac ctg gag agg acg agg cag ttc ctg gat tat    336
Lys Val Ser Ala Ser His Leu Glu Arg Thr Arg Gln Phe Leu Asp Tyr
            100             105             110


ctg cag gag ttc ctt ggt gtg atg agt aca gag tgg gca atg gaa ggc    384
Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp Ala Met Glu Gly
        115             120             125


taa                                                                 387
```

```
<210> 58
<211> 128
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitope

<400> 58
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
1               5               10                  15


Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20              25              30


Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
        35              40              45


Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
    50              55              60


Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
65              70              75              80
```

48

Lys Glu Lys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro
                    85   90   95

Lys Val Ser Ala Ser His Leu Glu Arg Thr Arg Gln Phe Leu Asp Tyr
            100   105   110

Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp Ala Met Glu Gly
        115   120   125

<210> 59
<211> 438
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitopes

<220>
<221> CDS
<222> (1)..(438)

<220>
<221> mutation
<222> (256)..(300)
<223> Tetanus toxoid P2 epitope

<220>
<221> mutation
<222> (301)..(363)
<223> Tetanus toxoid P30 epitope

<220>
<221> misc_feature
<222> (1)..(255)
<223> DNA encoding amino acids 1-85 of murine IL5

<220>
<221> misc_feature
<222> (364)..(435)
<223> DNA encoding amino acids 90-113 of murine IL5

<400> 59
atg gag att ccc atg agc aca gtg gtg aaa gag acc ttg aca cag ctg   48
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
  1   5   10   15

tcc gct cac cga gct ctg ttg aca agc aat gag acg atg agg ctt cct   96
Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
            20   25   30

gtc cct act cat aaa aat cac cag cta tgc att gga gag atc ttt cag   144
Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
        35   40   45

49

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ggg cta gac ata ctg aag aat caa act gtc cgt ggg ggt acc gtg gaa    192
Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
     50              55              60

atg cta ttc caa aac ctg tca tta ata aag aaa tac atc gat aga caa    240
Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
 65              70              75              80

aaa gag aag tgt ggc cag tac atc aag gcc aac tcc aag ttc atc ggc    288
Lys Glu Lys Cys Gly Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
             85              90              95

atc acc gag ctg ttc aac aac ttc acc gtg agc ttc tgg ctg cgc gtg    336
Ile Thr Glu Leu Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val
             100             105             110

ccc aag gtg agc gcc agc cac ctg gag agg acg agg cag ttc ctg gat    384
Pro Lys Val Ser Ala Ser His Leu Glu Arg Thr Arg Gln Phe Leu Asp
             115             120             125

tat ctg cag gag ttc ctt ggt gtg atg agt aca gag tgg gca atg gaa    432
Tyr Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp Ala Met Glu
     130             135             140

ggc taa                                                            438
Gly
145


<210> 60
<211> 145
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Murine Il-5
      modified by substitution with tetanus toxoid
      epitopes

<400> 60
Met Glu Ile Pro Met Ser Thr Val Val Lys Glu Thr Leu Thr Gln Leu
 1               5               10              15

Ser Ala His Arg Ala Leu Leu Thr Ser Asn Glu Thr Met Arg Leu Pro
             20              25              30

Val Pro Thr His Lys Asn His Gln Leu Cys Ile Gly Glu Ile Phe Gln
             35              40              45

Gly Leu Asp Ile Leu Lys Asn Gln Thr Val Arg Gly Gly Thr Val Glu
     50              55              60

Met Leu Phe Gln Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp Arg Gln
 65              70              75              80

Lys Glu Lys Cys Gly Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
             85              90              95

Ile Thr Glu Leu Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val
             100             105             110

Pro Lys Val Ser Ala Ser His Leu Glu Arg Thr Arg Gln Phe Leu Asp
             115             120             125
```

```
Tyr Leu Gln Glu Phe Leu Gly Val Met Ser Thr Glu Trp Ala Met Glu
   130               135               140
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Gly
145
```

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<210> 61
<211> 57
<212> DNA
<213> Homo sapiens
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<220>
<221> CDS
<222> (1)..(57)
<223> DNA encoding natural human IL5 leader sequence
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<400> 61
atg agg atg ctt ctg cat ttg agt ttg ctg gct ctt gga gct gcc tac    48
Met Arg Met Leu Leu His Leu Ser Leu Leu Ala Leu Gly Ala Ala Tyr
  1           5               10              15
```

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gtg tat gcc                                                        57
Val Tyr Ala
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```
<210> 62
<211> 19
<212> PRT
<213> Homo sapiens
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<400> 62
Met Arg Met Leu Leu His Leu Ser Leu Leu Ala Leu Gly Ala Ala Tyr
  1           5               10              15
```

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Val Tyr Ala
```

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<210> 63
<211> 60
<212> DNA
<213> Mus musculus
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```
<220>
<221> CDS
<222> (1)..(60)
<223> DNA encoding natural murine IL5 leader sequence
```

```
<400> 63
atg aga agg atg ctt ctg cac ttg agt gtt ctg act ctc agc tgt gtc    48
Met Arg Arg Met Leu Leu His Leu Ser Val Leu Thr Leu Ser Cys Val
  1           5               10              15
```

```
tgg gcc act gcc                                                    60
Trp Ala Thr Ala
            20
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<210> 64
<211> 19
<212> PRT
<213> Mus musculus

<400> 64
Met Arg Arg Met Leu Leu His Leu Ser Val Leu Thr Leu Ser Cys Val
 1   5   10   15

Trp Ala Thr Ala
          20

<210> 65
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Promiscuous T
      helper epitope

<400> 65
Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala
 1   5   10

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/24   A61K39/00   A61K39/385   A61K39/39   A61K31/70
       A61K48/00   C07K14/54   C12N1/21   C12N1/19   C12N5/10
       C12N15/70   C12N15/86   G01N33/68   A61P37/00   //A61K39/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EMBASE, WPI Data, PAJ, EPO-Internal, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 45448 A (BRESAGEN LTD.) 4 December 1997 (1997-12-04) cited in the application page 15, line 5 -page 16, line 2 claims --- -/--	1-7, 9-12,14, 15,17, 18, 21-25, 32-37, 61,62, 65-68

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 June 2000	29/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nooij, F

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Inter nal Application No

PCT/DK 00/00205

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 05849 A (MOURITSEN & ELSNER A/S) 2 March 1995 (1995-03-02) the whole document ---	1-7, 9-12,14, 15,17, 18, 21-25, 32-37, 61,62, 65-68
A	WO 98 17799 A (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA ET AL.) 30 April 1998 (1998-04-30) claims ---	27-31, 38-59
A	WO 97 00321 A (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 3 January 1997 (1997-01-03) examples claims ---	1-68
A	WO 98 47923 A (TANOX BIOSYSTEMS INC.) 29 October 1998 (1998-10-29) examples claims ---	1-68
A	WO 95 31480 A (S.P.I. SYNTHETIC PEPTIDES INC.) 23 November 1995 (1995-11-23) claims ---	1-68
A	WO 95 26365 A (UNITED BIOMEDICAL INC.) 5 October 1995 (1995-10-05) examples claims ---	1-53
A	K. TAKATSU: "Interleukin 5 and B cell differentiation." CYTOKINE AND GROWTH FACTOR REVIEWS, vol. 9, no. 1, March 1998 (1998-03), pages 25-35, XP002119733 the whole document ---	1-68
A	J. WELTMAN ET AL.: "Interleukin-5: a proeosinophil cytokine mediator of inflammation in asthma and a target for antisense therapy." ALLERGY AND ASTHMA PROCEEDINGS, vol. 19, no. 5, September 1998 (1998-09), pages 257-261, XP002119734 Province, RI, USA abstract ---	1-68

-/--

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	D. BROIDE ET AL.: "Intradermal gene vaccination down-regulates both arms of the allergic response." JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 99, no. 1 part 2, January 1997 (1997-01), page S129 XP002119735 St. Louis, MO, USA abstract 523 -----	27-31, 38-59

1

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9745448	A	04-12-1997	AU	2758497 A	05-01-1998
			EP	0904293 A	31-03-1999
WO 9505849	A	02-03-1995	AT	162723 T	15-02-1998
			AU	707083 B	01-07-1999
			AU	7009198 A	30-07-1998
			AU	7608094 A	21-03-1995
			CA	2170236 A	02-03-1995
			DE	69408342 D	05-03-1998
			DE	69408342 T	14-05-1998
			DK	752886 T	04-05-1998
			EP	0752886 A	15-01-1997
			ES	2112559 T	01-04-1998
			GR	3026419 T	30-06-1998
			JP	9505031 T	20-05-1997
WO 9817799	A	30-04-1998	AU	5002297 A	15-05-1998
			BR	9712852 A	16-11-1999
			EP	0958364 A	24-11-1999
WO 9700321	A	03-01-1997	AU	5991796 A	15-01-1997
WO 9847923	A	29-10-1998	AU	7132098 A	13-11-1998
WO 9531480	A	23-11-1995	AU	708472 B	05-08-1999
			AU	2441895 A	05-12-1995
			CA	2190494 A	23-11-1995
			EP	0759941 A	05-03-1997
			JP	10504018 T	14-04-1998
			US	5824483 A	20-10-1998
WO 9526365	A	05-10-1995	AU	2195395 A	17-10-1995
			CA	2186595 A	05-10-1995
			CN	1146772 A	02-04-1997
			EP	0811016 A	10-12-1997
			JP	9510975 T	04-11-1997